Molecular Microbial Source Tracking for the Little Venice Water Quality Monitoring Project


Enterococci

FIU SubContract 205002527

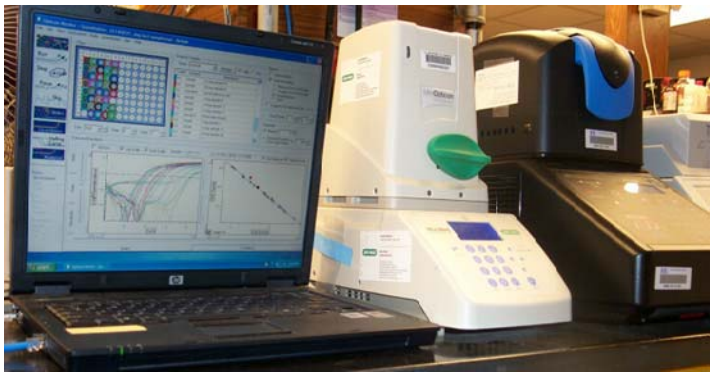
Final Report


Bacteroidales

Submitted to Florida International University

By the University of Miami Cooperative Institute for Marine and Atmospheric Studies

May 13, 2011



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FIU SubContract Number 205002527

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EXECUTIVE SUMMARY

The objective of the study reported here is to inform the Little Venice Water Quality Monitoring Project as to the relative detection, abundance and distribution of genetic host-source markers (and in particular human-host specific markers) for fecal indicator bacteria in the residential canals previously studied by the Little Venice Water Quality Monitoring Project. The Little Venice neighborhood was selected in the Monroe County Sanitary Wastewater Master Plan as the first phase of wastewater improvements for the Marathon area because of the large concentration of cesspools and inadequate septic systems, small average size of lots, high development density, and known water quality problems in the canals in the area. Little Venice includes the ocean side area of Vaca Key from Vaca Cut (east) to 94th Street (west), Marathon, FL. The Little Venice Service Area includes ~540 Equivalent Development Units (Fig. 1a, 1b).

The larger objectives of the over-all Little Venice Water Quality Monitoring Project are to detect changes in water quality as a function of remediation activities (Boyer and Briceño, 2006). This long-term assessment of the impacts of improved regional sanitary infrastructure upon local water quality of the Little Venice Service Area in the Florida Keys National Marine Sanctuary includes two phases: Phase I sampling (2001- 2003) was prior to remediation while Phase II (2005-2007) was the post-remediation stage sampling. The current molecular microbial source tracking study reported here is a follow-up to the Phase II post-remediation stage and includes sampling from 2009-2010. The initial experimental design was conceptually developed as a Before–After Control-Impact Design with multiple sites. Observations and sampling have been performed in three remedied canals (112th St., 100th St. and, 97th St. canals), including one canal with a stormwater discharge (100th St.), and in one canal lacking remedial actions still exposed to active septic fields (91st St. canal), plus an "offshore control site" for comparison purposes in the nearshore coastal waters approximately 100 meters offshore of the mouth of the 100th St. canal (Fig. 1a). Phase I was executed from May 23, 2001 to Dec. 15, 2003; Phase II began June 15, 2005, after the construction of the wastewater collection system was mostly completed. The Molecular Microbial Source Tracking (MST) study was conducted from Sept. 2009 to Sept. 2010.

Figure 1a: Satellite photo of sampling stations along residential canals of the Little Venice subdivision area in Marathon Key, Florida, as described in the prior Little Venice Study Water Quality Monitor Study report of Boyer and Briceño (2006). The sites sampled in this current report are the same as in the prior Little Venice WQ study and were sampled for MST markers and fecal indicators bi-monthly for one year, plus sampled for two seasonal 48-hour intensive diurnal studies

At a regional scale, natural water quality in the Little Venice area is the result of the dynamic interplay of complex natural settings with a man-modified landscape where driving processes are not constant but subject to trends and cycles of diverse periodicity and intensity. Marine currents exert an important influence on the distribution, character and interactions of water masses. The Florida Keys are highly interconnected by local and oceanic circulation patterns including Atlantic, Gulf and continental waters which in turn result in water quality diversity, both in time and space. At the local scale, the interaction is among water masses moving though Vaca Cut and along shore, ocean waters, runoff, ground waters and seepage from onsite sewage disposal systems. Water quality may be influenced with residence time in the canals and abundance of organic debris on their bottoms (Boyer and Briceño, 2006).

The prior studies by Florida International University during Phase I and II incorporated an assessment of microbial water quality utilizing traditional culture-based plate counts for total coliforms, fecal coliforms, and enterococci. However, the authors of the prior study were concerned that the lack of significant changes in the fecal indicator populations between Phase I and Phase II and between remediated and non-remediated canals might be due, at least in part, to a background environmental population of these fecal indicator bacteria from a combination of persistent environmental reservoirs and/or non-human host sources contributing to the culture-based measurements of total fecal coliforms or total enterococci, and confounding the assessment of remediation efficacy (Boyer and Briceño, 2006). A variety of molecular Microbial Source Tracking (MST) tools have recently been developed to specifically discriminate host sources of fecal indicator bacteria, and a variety of alternative fecal indicator microbes (including viral, bacterial, and protozoan source tracking markers) are being increasingly deployed to enhance the assessment of microbial water quality for coastal waters and recreational beaches (Solo-Gabriele

et al, 2011). Such molecular MST markers are well suited for identifying environmental host-sources of fecal indicators, to aid in relative risk assessment and to help guide microbial water quality remediation efforts. A suite of quantitative Polymerase Chain Reaction (qPCR) assays targeting a variety of MST markers, alternative fecal indicators, and pathogens, were utilized here in a follow-up MST study to the Phase II microbial water quality assessments for the Little Venice residential canals.

We report here the cumulative bacteriological data, including both traditional culture-based data and molecular microbial source tracking data, from the 9 selected stations within the Little Venice subdivision. Water was collected every other week from September of 2009 to September of 2010 for bacteriological analysis and enumeration of fecal indicators, including total culturable enterococci by EPA method 1600, total culturable Bacteroides by BBE plate counts, by quantitative PCR for total enterococci, total Bacteroidales, human-host-specific Bacteroidales, canine-host-specific Bacteroides, and human-host-specific Adenovirus, and by end-point PCR for presence/absence detection of human-host-source *Methanobrevibacter smithii* nifH gene, and for human-host-specific *Enterococcus faceium* esp gene. Field parameters collected during each sampling at each station included salinity, temperature, pH, dissolved oxygen (DO), and turbidity. In addition, two seasonal 48 hour intensive studies were conducted in Jan 2010 (i.e. "dry season"), and July 2010 (i.e. "wet season"). During each of these intensives, samples were collected every two hours for 48+ hours from the head and mouth of the 100th Street canal by ISCO autosamplers, and the samples were analyzed for the same parameters as the regular semi-monthly samples. Water samples were analyzed by UM-CIMAS personnel at the NOAA AOML laboratory in Miami by culture based and molecular based methodologies as outlined in our Quality Assurance Plan.

The remediated canals (112th St., 100th St., and 97th St.) showed lower overall averages of enterococci and of human source bacteroidales markers for semi-monthly sampling than did the septic control canal (91st St). The 91st septic control canal showed the greatest frequency of exceedances of enterococci regulatory limits, and the greatest frequency of detection and greatest average abundance of human source fecal markers for semi-monthly sampling, the remediated 112th St and 97 St canals showed the lowest frequency of regulatory exceedances and detection of human source markers, and the remediated 100th St. canal (which also had a stormwater discharge culvert at its mouth) showed an intermediate average for enterococci and human source marker abundance and frequency of detection for human source marker, with the greatest range of variability of the canals. Total bacteroidales by qPCR appeared to be ubiquitous and show frequent although variable elevations in all of the canals. In this case, total bacteroidales may not have been sufficiently informative to guide these remediation efforts. Dog-source marker was highly variable and was periodically seen in all canals and the offshore site, with no significant differences between remediated and septic canals for semi-monthly sampling. However, there was a sustained and significant cluster of substantially elevated abundance of dog fecal marker in all canals for a period of approximately one month from late April through May of 2010. During the course of the semi-monthly sampling, there was also an unusual outlier date, where anomalously high elevations were observed in all canals for enterococci and human source

bacteroidales, while concurrently there was no detection of any dog marker for any of the canals On this date, when extremely high levels of enterococci were detected by qPCR, both the 91st St. canal and 112th St. canal showed regulatory exceedances of viable enterococci by membrane filtration plate counts, and several canals showed detection of multiple human fecal markers, many at very elevated abundance more typically seen from samples such as wastewater outfalls. Even during this "outlier" event however, the 91st St septic control canal in particular showed the highest levels of human marker seen during the entire course of this study, and showed detection of all bacterial human-source markers that were tested for. The causes of this extreme but transient elevation in total enterococci and human fecal indicator markers are not clear. In two seasonal 48-hour diurnal intensive studies for the 100th St. Canal in the winter (dry) and summer (wet) seasons, significant variations were observed for the patterns of enterococci and source tracking markers. The winter season showed a significant increase in the frequency of regulatory exceedances of enterococci limits as compared to the summer season. In addition, the winter season showed a high frequency of low to moderately elevated human source marker as compared to the summer season where human marker was near or below the level of detection.

In summary, it does appear that in general the microbial water quality of the remediated canals was overall significantly improved in comparison to the non-remediated septic control canal, although the remediated canal with the stormwater discharge had a more intermediate and highly variable microbial water quality. This suggests that the remediation efforts on the sanitary infrastructure of the area have had a beneficial effect on changing patterns of fecal indicators and pathogens in the canals and nearshore waters, thus contributing to improved regional water quality. However, the patterns of dog-source marker, particularly during the summer "wet" season suggest that surface runoff and stormwater discharge may still be a significant source of negative impact on the nearshore microbial water quality of the region. The cyclical nature of low to moderate elevations for human marker during the winter 48 hour study suggest there may still be some persistent but low level source of human marker in these canals , and that this might be influenced by tidal cycle. The patterns of fecal indicators observed during the 48 hour studies and from the unusual "outlier" event date of Oct 27 2009 also suggest there are still other potentially transient sources for human fecal marker in these waters that may warrant further investigation.

A follow-up 48-hour diurnal MST study is anticipated to be conducted by the NOAA Atlantic Oceanographic and Meteorological Laboratory and the University of Miami Oceans and Human Health Center for the Little Venice canals during the summer of 2011. At the completion of the 2011 follow-up study, a supplemental report will also be delivered to Florida International University describing the additional results for summer of 2011.

ACKNOWLEDGEMENTS:

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BACKGROUND

(1) Water Quality History of the Little Venice Service Area:

(from Boyer and Briceño, 2006)

Since the early 1980's several Florida counties began monitoring beaches and canals for *Enterococci* (EC) and fecal coliforms bacteria (FC), because elevated concentrations of these bacteria were believed to be strongly correlated with the presence of human pathogens. Onsite disposal systems (OSDS) and injection wells are known to be a source of microbial contamination of groundwater (Keswick, 1984). Because the groundwaters and surface waters are very closely linked in the Keys, it is not surprising that fecal coliform bacteria are common in canals and boat basins (FDER, 1987).

The Little Venice neighborhood was selected in the Monroe County Sanitary Wastewater Master Plan as the first phase of wastewater improvements for the Marathon area because of the large concentration of cesspools and inadequate septic systems, small average size of lots, high development density, and known water quality problems in the canals in the area. Little Venice includes the ocean side area of Vaca Key from Vaca Cut (east) to 94th Street (west), Marathon, FL. The Little Venice Service Area includes ~540 Equivalent Development Units (Figures 1a, 1b, and figure 2). Water quality in the 89th – 91st Street canals was thoroughly studied in 1984-1985 as part of the Florida Department of Environmental Regulation's Monitoring Study (FDER, 1987). That study demonstrated significant nutrient enrichment of the canals, high Chlorophyll-a content, and high coprostanol concentrations in sediments. Coprostanol is a break-down product of cholesterol and has been used as an indicator of fecal contamination.

During year 2004 the Little Venice Service Area received a low-pressure, vacuum wastewater collection system to convey wastewater to a central treatment plant. The treatment plant produces effluents that meet or exceed the current advanced wastewater treatment (AWT) standards of 5:5:3:1 (BOD5, TSS, TN, TP) and uses a Class V injection well for disposal of treated wastewater. Central collection and treatment of wastewater removes a substantial portion of nutrient loading into the canals by removing the sources of wastewater (septic tanks and cesspits). As of October 2008, the ownership and management of the completed low pressure vacuum collection system and AWT treatment plant for the Little Venice Wastewater District was transferred from the Florida Keys Aquaduct Authority (FKAA) to the city of Marathon, Florida. Sewer implementation efforts continue in the Keys, and as of February 2011 about 15% of the larger Marathon Service Area is connected, which includes the Little Venice Service Area.

The objective of the Little Venice Monitoring project is to detect changes in water quality as a function of remediation activities. The initial experimental design was conceptually developed as a Before–After Control-Impact Design with multiple sites (BACI;

Eberhardt, 1976; Stewart-Oaten et al., 1986) and includes two phases. Phase, I from year 2001 to year 2003, corresponds to the pre-remediation stage, and Phase II, which began in 2005 after the construction of the wastewater collection system, is the post-remediation phase. Four canals within the Little Venice Service Area were selected for study (Figures 1a, 1b). The first canal is a connected "U-shaped" canal system located at 112th Street, lined with single-family residences that were constructed prior to 1970. A high percentage of those residences had inadequate sewage treatment systems. The second canal is located adjacent to 100th Street and the third one is located adjacent to 97th Street. Both are dead-end canals that are lined with single-family houses and mobile homes. Many of these residences had poorly functional septic systems or cesspits. Finally, the 91st Street canal was been selected as a reference canal not subjected to remediation measures during the time of the study. It is located west and outside the Little Venice Service Area.

Starting in June of 2005, the Southeast Environmental Research Center of Florida International University commenced a Phase II sampling and analysis program of the 9 stations shown in Figure 1a. The approach of this study was primarily to assess nutrient loading and physical parameters but some assessment of bacteriological water quality was also conducted by standard culture-based methods for Fecal Coliforms (SM 9222D) and Enterococci (EPA method 1600). Results of this Phase II sampling are reported in Boyer and Briceño (2006). This prior study found that the head of the canals had greater bacterial numbers than the mouth, as would be expected because of tidal mixing with offshore waters, and that most stations displayed a similar pattern with maxima centered about July-September and December-January, a persistent minimum in March-May and a more subdued minimum in November. This study further found that there were not statistically significant changes between Phase I and Phase II sampling or between canal sites for the occurrence of regulatory exceedance events for fecal indicator abundance, either for fecal coliforms or enterococci. However fecal coliforms abundance in the whole data set did show a significant decrease after remediation, and fecal coliform counts at all canal heads and at the mouth of Canal 112th St. also experienced significant decreases. Despite this, the changes observed for enterococci were non-significant, except for a slight but significant increase at the offshore site.

Survival, persistence, and even regrowth of fecal coliforms and enterococci fecal indicating bacterial (FIB) have been well documented in the literature for a wide variety of environmental habitats and substrates, including a variety of soils, beach sands, subtropical and tropical marine sediments and soils, macrophyton detritus, and epiphytic biofilms on terrestrial, aquatic, and marine plants (Anderson, 1997, 2005; Solo-Gabriele et al., 2000; Rozen and Belkin, 2001; Yamahara et al., 2007; Hartz, 2008; Ishii et al 2006; Boehm, 2009; Signoretto et al., 2004; Ferguson and Signoretto, 2011). The authors of the prior Little Venice study were concerned that the lack of significant changes in the fecal indicator populations between Phase I and Phase II and between remediated and non-remediated canals might be due, at least in part, to such a situation where there may be a background environmental population of these fecal indicator

bacteria contributing to the culture-based measurements of total fecal coliforms or total enterococci. The traditional culture-based methods currently used for regulatory monitoring do not have a source-tracking capability and cannot discriminate between host-sources or environmental background populations of FIBs. The authors were concerned that immediate remediation results for fecal coliforms and *Enterococci* in the Little Venice canals may be masked by their re-growth in organic-rich (nutrient-rich) debris on the canal bottom or supplied by alternative sources as runoff, especially from storm action, and because of this they no longer considered the traditional culture-based methodology an unbiased index. Therefore, to address and clarify this issue, the follow-up microbial source tracking study described here in this current report was initiated by the University of Miami Cooperative Institute for Marine and Atmospheric Studies and the National Oceanic and Atmospheric Administration Atlantic Oceanographic and Meteorological Laboratory from Sept 2009 to Sept 2010. The goals of this MST study were to determine if there were any substantial differences between the remediated and non-remediated canals for molecular markers of enterococci, alternative fecal indicator bacteria (Bacteroidales), or host-source fecal markers (particularly human-specific fecal markers) that might indicate positive remediation effects potentially masked when assessed only by traditional culture-based microbial water quality methods.

At a regional scale, natural water quality in the Little Venice area is the result of the dynamic interplay of complex natural settings with a man-modified landscape where driving processes are not constant but subject to trends and cycles of diverse periodicity and intensity. Marine currents exert an important influence on the distribution, character and interactions of water masses. The Florida Keys are highly interconnected by local and oceanic circulation patterns including Atlantic, Gulf and continental waters which in turn result in water quality diversity, both in time and space. At the local scale, the interaction is among water masses moving across Vaca Cut and along shore, ocean waters, runoff, ground waters and seepage from cesspits. Water quality changes with residence time in the canals, which in turn varies according to canal geometry (i.e. straight versus U-shaped), canal seaward extension (i.e. 97th St. canal), bottom topography, accumulation of organic debris and tide and wind intensities, among other factors. These organic-rich debris pools, where bacteria thrive, are stirred back and forth during tides and are incorporated in the water column (Boyer and Briceño, 2006)..

(2) Limitations of Traditional Culture-Based Methods for Enumeration of Fecal Indication Bacteria in Environmental Samples: (adapted in part from: Hagedorn et al, 2011)

Establishing the safety of recreational waters is currently based upon measurements of "indicator" microbes, which are generally bacteria but may also be viruses or protozoa. These microbes are used as "indicators" of the possible presence of sanitary sewage and subsequent risks to human health. Indicator microbes are natural inhabitants of the gastrointestinal tract of humans and are present in large numbers in fecal releases, especially releases from humans and

warm-blooded animals (Maier et al. 2008). Indicator microbes are not necessarily pathogenic but are used as surrogates for the presence of pathogenic microbes. For marine waters, enterococci are the indicators recommended by the US Environmental Protection Agency (US EPA). Two criteria have been identified by the US EPA for regulatory purposes: One based upon geometric mean density and the other based upon single sample maximums (US EPA 1986). For marine waters, the acceptable geometric mean level (35 "colony forming units," or "CFU," enterococci per 100 mL of water) is independent of the intended use of that water, whereas the single sample maximums are regulated depending upon the intended use of the recreational area (designated beach area = 104 CFU/100 mL, moderate full body contact = 158 CFU/100 mL, lightly used full body contact = 276 CFU/100 mL, and infrequently used full body contact = 501 CFU/100 mL). In most cases, the 104 CFU/100 mL standard is used. Prior epidemiologic studies conducted at beach locations impacted by point sources of pollution have established relationships between indicator bacterial levels in water and human disease (Cabelli 1979, 1982), given the high probability of finding both fecal indicator bacteria (FIB) and human pathogens in waters impacted by point sources of pollution. In these cases, the levels of fecal bacteria "indicate" a risk to public health. In the developed world, wastewater treatment and effluent disposal systems continue to be upgraded by communities, thereby diminishing the dominance of point sources of pollution at beaches and coastal water bodies. Nevertheless, beaches and coastal water bodies worldwide are still impacted by fecal pollution, even though microbial contributions to many of the recreational water bodies are no longer dominated by sewage from ocean outfalls and compromised sewage pipes. Rather, the sources of microbial pollution at many sites, particularly in developed countries, are now predominantly "nonpoint" sources, which include contributions from human bathing activities (as opposed to collective fecal contributions from a large community), from maritime and recreational boating sources (such as "live-aboards"), diffuse residual contamination from decommissioned legacy septic fields, from animal sources (such as birds, dogs, livestock, and wild animals), from urban and stormwater runoff, and from natural sources (e.g., from persistence and regrowth of indicators in the environment) (Bernhard and Field 2000; Wright et al. 2009, 2011; Desmarais 2002). Thus, at nonpoint-source-impacted coastal environments such as beaches, residential canals, coastal inlets and near-shore coastal waters typical of the Florida Keys, fecal indicator microbes can originate from many different sources.

Currently, regulatory methods for assessing microbial water quality in recreational and shellfishing waters is based on culture methods targeting enterococci, Escherichia coli, and/or fecal coliforms. The States set the specific water quality regulations for a particular area, typically based on recommendations by the US EPA, although some state may expand or supplement the EPA recommendations for regulatory purposes. For example Hawaii also uses *Clostridium perfringens* as an alternative fecal indicator, and many States continue to use E coli and/or fecal coliforms for ambient marine waters in addition to enterococci. In the case of Florida, the State sets regulatory limits for fresh and marine recreational waters based on enterococci and Escherichia coli following EPA recommended exposure limit guidelines (for

enterococci 104cfu/100mL for single grab samples or 35 cfu/100mL for geometric mean). However, EPA currently only recommends culture-based enterococci for assessment of marine recreational waters. There are two culture based methods for measuring enterococci that are currently approved for regulatory purposes: membrane filtration plate counts on mEI agar (EPA Method 1600), and a commercial Most Probable Number test by the IDEXX company based on chromogenic substrate (IDEXX EnteroLert™). Both of these tests require incubation of approximately 24 hours, do not have any ability to discriminate host sources, and do not measure the total population including dormant or viable cells not in a culturable state, but only the culturable sub-population of enterococci in an environmental sample. The statistical and predictive relationships between indicator microbes such as enterococci and pathogens originating from nonpoint sources of fecal pollution are different (less direct) than those between indicators and pathogens present in point sources of pollution. This situation complicates interpretations of indicator microbe data for beaches and coastal waters impacted by nonpoint source pollution, particularly with respect to understanding relationships to public health (WERF 2009). Since the culture based methods currently in use cannot discriminate host sources, the assessment of relative risk of detected fecal contamination can be problematic, as different host sources are believed to have significantly different risk to human and ecosystem health, with fecal contamination from human populations and agricultural livestock thought to pose the greatest risk. In addition, current culture based methods cannot discriminate changing patterns of host sources when assessing potential improvements due to sanitary infrastructure remediation. Molecular Microbial Source Tracking markers are well suited for identifying sources of indicator bacteria. As such, MST represents a valuable tool for regulators who wish to identify the source of an indicator microbe signal for purposes of interpreting the public health implications and ultimately remediating the source altogether.

(3) Molecular Microbial Source Tracking of Fecal Indicator Bacteria and Fecal Pathogens to Enhance Traditional Culture-Based Microbial Water Quality Assessments: (Adapted in part from: Hagedorn et al, 2011)

Microbial source tracking (MST) is a new and emerging sub-discipline of environmental microbiology that allows practitioners to discriminate among the many possible sources of fecal pollution in environmental waters. MST's current and potential applications range from beach monitoring to total maximum daily load (TMDL) assessment of pollution sources, that in turn will mediate greater protection of public health and improvement of environmental water quality. The identification of the fecal sources is important to protect the public from zoonotic pathogens that may be shed by animals such as wild birds, poultry, cattle, and pigs. The capability to detect human-source pollution is also crucial to management strategies, as sewage and septic discharges from human origin are generally expected to have a higher risk to public health than that of animal origin. Consequently, understanding the origin of fecal pollution is essential in assessing

potential human health risks as well as for determining the actions necessary to remediate the quality of waters contaminated by fecal matter.

A range of bacterial source tracking techniques is grouped under what is commonly referred to as library-dependent methods . These methods require the construction of a library of known source profiles that are used for comparison with environmental isolates to determine sources of contamination. These fecal indicator library profiles may be based on genetic sequence data, gene expression profiles, antibiotic resistance profiles or biochemical metabolic profiles, but in most cases, these population profiles may be geographically and/or temporalIy specific, thus requiring specific community libraries be developed for each habitat to be assessed by library-dependent MST methods.

In recent years numerous library-independent methods for microbial source tracking have become available either relying on selective cultivation of source-specific bacteria or, increasingly, on direct detection of source-specific genetic markers. This is the MST approach utilized in the work we report here. Numerous other successful MST applications have proven the practicality and potential of library-independent bacterial MST methods for the characterization and identification of fecal pollution sources. A variety of alternative fecal indicator species and host-specific gene targets for library-independent MST have been proposed and tested in recent literature. Enterococci have proven to be problematic for host source tracking, and a recent study showed that no single species of *Enterococcus* seems to be indicative for a specific source, but assemblages as determined by multiplex PCR applied on *Enterococcus* enrichment cultures may support source identification to some extent (Layton et al, 2010). A human-associated enterococci marker for a human-specific enterococcal surface protein (esp) gene of *Enterococcus faecium* has been developed and deployed for several studies (Scott et al, 2005, Ahmed et al, 2008), however there has been some debate about its specificity and efficacy. This esp marker is found in a relative minority of human population so may function best to discriminate large combined fecal inputs representing a sizable human populations, such as municipal sewage discharge, but may not be as effective at detection from individuals or small scale inputs (i.e. individual septic field, etc.) Detection of enterococci esp marker is also more sensitive when combined with pre-enrichment of sample by culture.

Methods that do not rely on detection based on growth of bacterial populations or isolated bacteria present in a sample have become increasingly widespread in recent years and mostly target either the 16S rRNA gene or sequences obtained from metagenomic fragments. They usually involve target enrichment by filtration followed by extraction of nucleic acids and storage at low temperature prior to amplification of target genes by PCR or qPCR. So far, members of the order *Bacteroidales*, the genera *Methanobrevibacter*, *Rhodococcus*, *Faecalibacterium*, *Catellicoccus*, pathotypes of *Enterococcus* and *Escherichia*, and the bifidobacteria have been reported as being associated with specific animal hosts or pollution sources (Wuertz et al, 2011). The order *Bacteroidales* is currently the most widely used taxon targeted for source identification for livestock (pigs, cattle, sheep, horses, and chicken) and

domestic pets (dogs and cats), and *Bacteroides* is considered the predominant genus of human fecal bacteria (Holdeman et al. 1976). The prevalence of *Bacteroidales* in the human gut makes human-associated *Bacteroidales* assays highly sensitive. The marker BacHum-UCD (Kildare et al. 2007) makes up on average 82% of total *Bacteroidales* in human guts detected by the general BacUni assay (Silkie and Nelson 2009). Thus, it is easily detectable if there is human fecal contamination present in water samples.

One major difference between culture methods and molecular methods targeting fecal indicator genes in environmental samples is that these two approaches do not measure the same population of target fecal indicator organisms, but rather culture methods only enumerate viable cells, while the molecular methods (at least at present) cannot discriminate between viable, dormant, or dead cells. PCR or qPCR MST methods based on genomic DNA detects both viable cells and dead cells; even particle-attached DNA might be targeted. For those cases where PCR/qPCR results have to be compared with results from culture-based approaches, they tend to overestimate the number of viable bacterial cells. A variety of approaches are currently being investigated to improve discrimination of viable cells by molecular methods and show great future promise, such as treatment of samples with propidium monoazide or ethidium monoazide to degrade DNA in cells that have compromised membranes, but these approaches are still in the developmental stages with numerous caveats and limitations. Thus in the study reported here, the qPCR based MST assays do not distinguish between live and dead target cells.

For the purposes of this Little Venice MST study, qPCR based MST assays were chosen to discriminate human-source Bacteroidales, dog-source Bacteroides, total general Bacteroidales, a human-associated enterococci surface protein gene (esp), human-associated Methanobrevibacter smithii, and a human pathogen adenovirus marker. A primary goal of this study was to enhance the culture based data for viable enterococci relative abundance with an assessment of changes in the abundance and frequency patterns of human-associated fecal markers between remediated and non-remediated residential canals in the Little Venice Service Area.

ANALYTICAL METHODS

Field Sampling:

Water from the nine sample stations shown in Figure 1b were sampled on a semi-monthly basis from Sept 1, 2009 through Sept 4 2010. Water was collected from just below the surface in sterile 2.4L polypropylene bottles and stored on ice or cold gel packs in cooler until return to the

Laboratory and processed within 6 hours of collection. Canal sites were collected from alongshore the canals, and the offshore control site # 2 was collected from a kyak (launched from site # 4). Physical parameters including salinity, temperature, dissolved oxygen, pH, and turbidity was measured in situ at the time of collection, utilizing a YSI multimeter and probes, along with a turbidometer, provided by Florida International University (Table 1).

48+ hour diurnal sampling covering multiple tidal cycles of the mouth and head of the 100th St. canal was also conducted for two discrete seasons, utilizing ISCO autosamplers equipped with YSI datasondes (also provided by Florida International University). The winter ("dry season") diurnal sampling was conducted from10:00 hours EST on 1/26/2010 to 10:00 hours EST on 1/28/2010, and the summer ("wet season") diurnal sampling was conducted from 13:00 hours EST on 7/19/2010 to 07:00 hours EST on 7/22/2010. Two ISCO autosamplers each at the head and mouth of the 100th St. canal (4 ISCO samplers total) were programmed to collect 4L water samples every 2 hours for the duration of the diurnal study. ISCO autosampler bottles were maintained on ice in the instrument during the autosampler run and were retrieved for processing every 4 hours (or 6 hours for the overnight run), and returned to the local Keys Marine Lab (Florida Institute of Oceanography, town of Layton, Long Key, Florida) for processing. YSI datasondes accompanying the ISCO autosamplers measured and recorded physical parameters in situ (salinity, temperature, dissolved oxygen, pH) on an hourly basis throughout the duration of the diurnal study.

Laboratory Analysis:

Upon return of the water samples to the Laboratory (either NOAA-AOML for semi-monthly sampling or KML on Long Key for 48hour diurnal studies), 100mL water samples were filtered on cellulose nitrate filter discs (47mm, 0.45micron, Whatman) and plated for mEI agar plate counts of viable enterococci according to EPA method 1600. To assess potentially viable Bacteroidales, 100mL water samples were also filtered onto cellulose nitrate filters and incubated on Bacteroides Bile Esculin (BBE) agar under anaerobic conditions using GasPak EZ Anaerobe Incubation Pouches (BD) as previously described (Sinigalliano et al., 2010)

For community DNA extractions, 1 liter water samples were filtered through cellulose nitrate filters (47mm diameter, 0.45 micron, Whatman), then aseptically rolled and placed into 2mL beat-beat lysing matrix tubes (MPBiomedicals), and stored frozen at -80°C until subsequent processing and extraction. For later extraction of DNA, frozen filters were spiked with a known number of washed whole cells of *Lactococcus lactis* quantitative extraction control culture, lysed and extracted by bead beating in a FastPrep FP120 instrument, and purified with the FastDNA Spin Kit (MPBiomedicals), all as previously described (Sinigalliano et al., 2010). The purified DNA was ultimately eluted in 100uL final volume. Extract elutions were divided into replicate aliquots and stored frozen at -80°C until subsequent molecular analysis.

For community RNA extractions, 1 liter water samples were first adjusted to pH 3.5 to enhance adsorbtion of viral particles to charged filters, then filtered through HA type membrane filters (47mm diameter, 0.45micron, Millipore). Filters were aseptically rolled and placed in 2mL beat-beat lysing matrix tubes (MPBiomedicals), and stored frozen at -80°C until subsequent processing and extraction. Later, viral DNA and RNA was simultaneously extracted directly from the HA filters using the QIAamp MinElute Virus Spin Kit (Qiagen) as per manufacturer's directions with the following exceptions: Filters in beat-beat lysing matrix tubes received 50uL of Qiagen Protease, 400uL of sterile 0.9% NaCl solution, and 400uL of Qiagen Buffer AL. Lysis tubes were then spiked with a known number of *Lactococcus lactis* quantitative extraction control washed whole cells, then the lysis tubes were bead beat in a FastPrep FP120 instrument for 30 seconds at the 5.5 speed setting. The bead beat tube was centrifuged for 1 min at ~14,000rpm to pellet filter and cell debris, beads, etc. and the supernatant lysate was transferred to a fresh sterile 2mL microfuge tube. The entire contents of the lysate were passed through the QIAamp MinElute Virus Spin Filter, using sequential spins until all lysate had been loaded onto the purification spin filter, which was then processed and purified according to the Kit directions, and the purified nucleic acids were ultimately eluted in 100uL final volume. Extract elutions were divided into replicate aliquots and stored frozen at -80°C until subsequent molecular analysis.

All extracts were subsequently analyzed by quantitative real-time polymerase chain reaction (qPCR) by "Taqman" type 5'-exonuclease probe chemistry using a Chromo4 four-color real-time qPCR thermocycler (BioRad/MJResearch) for the following assays:

1) Total enterococci by the EPA qPCR assay "entero1" (Haugland et al, 2005, Siefring et al, 2008)

2) Total general Bacteroidales by the EPA qPCR assay for AllBac "GenBac3" (Siefring et al, 2008)

3) Human-source-specific Bacteroidales by the qPCR assay "BacHum-UCD" (Kildare et al, 2007)

4) Human-source-specific Bacteroidales by the EPA qPCR assay "HF183" (Shanks et al, 2009, Haugland et al, 2010)

5) Human viral pathogen Adenovirus by the hexon gene qPCR assay "JTVXP" (Jothikumar et al, 2005)

6) Dog-source-specific Bacteroides by qPCR assay "AOML-DogBact" (Sinigalliano et al, 2010, Shah et al, 2011)

(Note the original project proposal also included a human Polyomavirus qPCR assay, however this assay had to be dropped because appropriate standard reference material could not be acquired for the duration of the project).

All extracts were also analyzed for presence/absence of targets by non-quantitative end-point PCR for the following assays:

1) Human-source-specific Methanobrevibacter smithii by the *nifH* gene PCR assay "M.smithii-nifH" (Johnston et al, 2010) – *note this was done as end-point PCR for this particular Little Venice study rather than by qPCR for lack of availability of standard curve reference material at the time of study. Followup studies are now using this assay in a qPCR format.*

2) Putative human-source-specific enterococci by the *esp* gene PCR assay "Ent-esp" (Scott et al, 2005; Ahmed et al, 2008)

For all molecular assays, the qPCR assay conditions and detailed methodology, including primer and probe sequences, reagent concentrations, thermocycling conditions, extraction controls, inhibition controls, standard curve generation, etc. were as previously described for entero1, GenBac3, BacHum-UCD, Ent-esp, and AOML-dogBact (Sinigalliano et al, 2010, Shah et al, 2011), for HF183 (Shanks et al 2009), for human Adenovirus (Jothikumar et al, 2005), and for human methanobrevibacter (Johnston et al, 2010).

RESULTS

Table 1 summarizes the overall results of the study for all data. This includes fecal indicator and host-source marker detection and enumeration for all sample sites during the course of the study, as well as culture-based enumeration of enterococci and Bacteroides, and physical parameters. We do not show here the results of the concurrent nutrient analysis as conducted by Florida International University.

There are a few data gaps in the study data archive as follows: (1) Molecular results are missing for the dates of 9/8/2010 and 9/15/2010 due to an instrument failure in batch processing that rendered unacceptable extraction recovery efficiencies for the samples from these two dates; (2) YSI sonde measurements are missing for 7/7/2010 as the instrument provided by FIU was not available for that date, and no replacement instrument was available; (3) turbidity measurements are missing for 6/8/2010, as the instrument provided by FIU was not available for that date; (4) dissolved oxygen measurements are missing for the 7/27/2010 due to an instrument error with the probe; and (5) during the summer diurnal 48 hour study there are two time points missing (the 01:00 and 03:00 hour timepoints of 7/20/2010 respectively) due to a programming error of the ISCO autosamplers – however additional timepoints were added to the end of this diurnal sampling to extend it to 07:00 on 7/22/2010.

Enterococci as enumerated by mEI plate counts (EPA method 1600) showed seasonal and geographic variation during the semi-monthly sampling as seen in Table 1, and Table 2. The remediated canals of 112th St, 100th St, and 97th St showed lower overall averages for the semi-monthly sampling than did the 91st St septic control canal, as well as a lower frequency of exceedance events for the regulatory exposure limits for enterococci. The 91st St septic control canal had the highest average abundance viable enterococci and frequency of exceedance events, while the remediated 112st St and 97th St had the lowest. The remediated 100th St canal had a intermediate average enterococci abundance and frequency of exceedance, and also the greatest variability in these measures. However, it should be remembered that the 100th St canal also contains a stormwater discharge culvert at the head of the canal that drains a substantial area across US highway 1 in the vicinity of the Marathon Airport, and this canal may thus be particularly sensitive to both surface runoff and directed stormwater discharge.

Total Enterocooci as enumerated by the “entero1” qPCR assay also showed seasonal and geographic variation during the semi-monthly sampling, roughly mirroring the general trends of the viable enterococci, as seen in Tables 1-3, Figure 3, and Figures 7-9. Again the 91st St septic control canal showed a greater average abundance of enterococci, greater frequency of detection, and greater frequency of regulatory exposure limit exceedance events as compared to the

remediated canals, while the 100th St canal with the stormwater discharge culvert showed the greatest variability.

Viable Bacteroides, as measured by anaerobic culture on BBE media, were generally low and on average did not show significant differences during the semi-monthly samplings between the remediated and non-remediated canals (Table 1). Total general Bacteroidales as measured by qPCR, were relatively ubiquitous in most samples during the semi-monthly samplings and were frequently either "elevated" (>100 GE/100mL) or "substantially elevated" (>1000 GE/100mL) for most sample dates and sample sites, including the offshore control site #2 with no substantial difference in overall average abundance or frequency of detection between remediated and non-remediated canals (Table 1, Table 4, Figure 4). However the 91st St septic control canal did show an increased frequency of "substantial elevations" of total general Bacteroidales as compared to the remediated canals (Table 2). Given the relatively ubiquitous nature of the detection of this marker for this region, total general Bacteroidales may not prove to be particularly informative as regards the efficacy of sanitary remediation efforts for this area.

Human-host-specific Bacteroidales, as measured by both the BacHum-UCD and HF183 qPCR assays, showed both greater average overall abundance, greater frequency of detection, and greater frequency of "substantial elevation" for the 91st septic control canal during the semi-monthly sampling than for the remediated canals, although the 91st St canal also showed significantly greater range of variability for human-source Bacteroidales (Tables 1-3, Figures 5-6). Elevations of human-source Bacteroidales abundance were more frequent during the drier season from September through March. This was also observed during the two seasonal 48hour diurnal studies (Figures 14-15), where the winter ("dry season") 48 hour study showed significantly greater detection frequency and abundance of human-source Bacteroidales than did the summer ("wet season") 48 hour study where these human fecal markers were near or below detection limit for most of the samples.

Detection of human-specific Enterococci esp gene marker was extremely rare during the study, but was most frequently detected in the 91st St septic control canal (Table 1 and Table 3). Detection of the human-specific *Methanobrevibacter smithii* nifH gene marker was also rare during the study, but did not appear to differ significantly between canals (Table 1 and Table 3).

Detection of dog-specific Bacteroides marker was variable in both abundance and in spatial/temporal distribution, and was not distinguished between remediated vs non-remediated canals. Both the 91st St septic control and the 112th remediated canal showed similar frequencies of detection and relative overall average abundance, but all sample sites (including the offshore control sites) showed periodic elevations of this dog fecal marker (Tables 1-3, Figures 10-11). Detection of dog-specific Bacteroidales during the semi-monthly sampling did show a specific clustering of dates for detection events, particularly with a sustained cluster of "substantially elevated" dog fecal marker observed in all canals from late April through May of 2010 (Figure 11). The seasonal pattern of elevation for dog-specific fecal marker is quite different than that

observed for human-specific fecal maker, with wide-spread detection of dog marker during the summer "wet season" which experiences significantly greater surface run-off and stormwater discharge. The overall abundance of dog fecal marker, when detected, was also typically orders of magnitude higher than that seen for human marker (with the exception of the "outlier date", see below). For the most part, detection of human marker was relatively rare and of relatively low abundance for most samples, while dog marker (when it was detected) was frequently substantially elevated. We hypothesize that this wide-spread regional pattern of detection for dog marker in the canals and the offshore site may relate to surface runoff and/or stormwater discharge during the rainy season.

During the course of the semi-monthly sampling there was a particular date, October 27, 2009, which showed extremely high levels of abundance for many of the markers from most of the sample sites. However, within this sampling date there are still geographic variations and all quantity control blanks and standards are as normal, so these anomalous measures, representing outlier data for most of the markers (including both culture and molecular assays), are not artifacts but reflective of actual, if anomalous, conditions on this particular "outlier date" (Table 1, Figure 15). High elevations were observed in all canals for enterococci and for human source Bacteroidales, while concurrently there was no detection of any dog marker for any of the canals On this date, both the 91st St. canal and 112th St. canal showed regulatory exceedances of viable enterococci by membrane filtration plate counts, and several canals showed detection of multiple human fecal markers, many at very elevated abundance levels more typically seen from samples such as wastewater outfalls. Even during this "outlier" event however, the 91st St septic control canal in particular showed the highest levels of human marker seen during the entire course of this study, and showed detection of all of the bacterial human-source markers tested. The causes of this extreme but transient elevation in total enterococci and human fecal indicator markers are not clear.

For the two seasonal 48-hour diurnal studies, with sampling every two hours, significant variations in the patterns of several markers were seen, both between head and mouth of the canal, and between wet and dry season (Table 1, Figures 12-15). The winter "dry season" showed a significant increase in the frequency of regulatory exceedances of enterococci exposure limits as compared to the summer "wet season" (Figures 12-13). The winter season also showed a higher frequency of moderate elevations of human-source fecal marker as compared to the summer season, when human marker by contrast was near or below detection limit (Figures 14-15). Abundance of human marker during the winter 48hour study was substantially higher at the head of the canal than at the mouth and showed cyclic pattern of peaks of human marker at timepoints of 20:00-22:00 hours, 10:00 hours, 16:00 hours, 20:00-22:hours, and 08:00hours respectively (Figure 14). A similar cyclic pattern of peaks for enterococci abundance was also observed for about these same timepoints for total enterococci (Figure 12). This pattern of cyclic abundance for enterococci and human Bacteroidales during the winter 48hour study may very roughly relate to the pattern of outgoing tidal cycle, however it does not appear to be a very good

fit to the tidal stage pattern (Figure 12). Conversely, the summer season showed significantly greater frequency and abundance of dog-source fecal marker with almost no human marker detection, but the abundance of dog-source Bacteroides appears greatest at the mouth of the canal rather than the head, and it does not show a similar cyclic pattern over the 48 hours as does the human marker, but rather appears clustered in the time frame between 21:00 hours on 7/20/2010 and 15:00 hours on 7/21/2010 (Table 1, Figure 15). The pattern of this cluster starts off with an initial high peak and slowly drops in abundance over time, consistent with the hypothesis that this might represent some type of concentrated dog fecal contamination event on this date during the summer 48hour study, that is slowly diluted and washed out over time (Figure 15), whereas the winter 48hour study human marker pattern appears to be re-current (although of relatively low abundance) during the course of the 48 hour observations (Figure 14).

Conclusion:

In summary, it does appear that in general the microbial water quality of the remediated canals was overall significantly improved in comparison to the non-remediated septic control canal, although the remediated canal with the stormwater discharge had a more intermediate and highly variable microbial water quality. This suggests that the remediation efforts on the sanitary infrastructure of the area have had a beneficial effect on changing patterns of fecal indicators and pathogens in the canals and nearshore waters, thus contributing to improved regional water quality. However, the patterns of dog-source marker, particularly during the summer "wet" season suggest that surface runoff and stormwater discharge may still be a significant source of negative impact on the nearshore microbial water quality of the region. The cyclical nature of low to moderate elevations for human marker during the winter 48 hour study suggest there may still be some persistent but low level source of human marker in these canals , and that this might be influenced by tidal cycle. The patterns of fecal indicators observed during the 48 hour studies and from the unusual "outlier" event date of Oct 27 2009 also suggest there are still other potentially transient sources for human fecal marker in these waters that may warrant further investigation.

A follow-up 48-hour diurnal MST study is anticipated to be conducted by the NOAA Atlantic Oceanographic and Meteorological Laboratory and the University of Miami Oceans and Human Health Center for the Little Venice canals during the summer of 2011. At the completion of the 2011 follow-up study, a supplemental report will also be delivered to Florida International University describing the additional results for summer of 2011.

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TABLES

Table 1: Summary of Raw Data from Little Venice MST Study Sept 2009 – Sept 2010

Date	Time	Sample ID Label	culturable Enteroccoci by MEI plate count CFU/100ml	Culturable Bacteroides by BBE plate count CFU/100ml	total Enterococci by entero1 qPCR GE/100ml	Human-specific Bacteroidales by BacHum-UCD qPCR GE/100ml	Human-specific Bacteroidales by HF183 qPCR GE/100ml	human-specific Methanobrevibacter smithi by nifH gene presence/absence endpoint PCR	human-specific enterococci by esp gene presence/absence endpoint PCR	human-specific adenovirus by hexon gene qPCR TSC/100ml	Dog-specific Bacteroidales by AOML DogBac qPCR TSC/100ml	Total Bacteroidales by AllBac - GenBac3 qPCR GE/100ml	salinity ppt	temp C	DO mg/l	pH	turbidity
9/1/2009	10:30	MST-LV01-01	14	1	29	0	0	(-)	(-)	0	71	3088	36.48	32.3	3.76	7.85	*
9/1/2009	10:00	MST-LV01-02	5	6	21	0	0	(-)	(-)	0	0	3492	36.16	31.1	4.32	7.96	3.94
9/1/2009	10:40	MST-LV01-03	20	0	27	0	0	(-)	(-)	0	0	6834	36.1	31.1	4.32	7.96	3.94
9/1/2009	9:47	MST-LV01-04	18	5	27	0	0	(-)	(-)	0	0	3716	36.52	32.12	3.39	7.93	1.98
9/1/2009	10:10	MST-LV01-05	17	1	15	0	0	(-)	(-)	0	0	3292	36.37	32.15	3.65	7.84	1.36
9/1/2009	9:30	MST-LV01-06	26	7	25	1	0	(-)	(-)	0	0	2218	36.24	32.15	3.97	7.96	2.99
9/1/2009	9:40	MST-LV01-07	8	5	0	2	0	(-)	(-)	0	0	*	36.13	32	3.96	7.92	3.23
9/1/2009	8:30	MST-LV01-08	50	22	80	0	0	(-)	(-)	0	6	4150	36.03	31.84	3.73	7.92	3.46
9/1/2009	9:15	MST-LV01-09	15	1	32	0	0	(-)	(-)	0	12	55	36.41	31.96	3.97	8.06	3.71
9/8/2009	10:17	MST-LV02-01	5	29	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	37.17	30	2.99	7.76	1.49
9/8/2009	10:10	MST-LV02-02	4	59	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	37.15	29.9	3.49	7.85	2.46
9/8/2009	10:31	MST-LV02-03	16	61	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	37.02	30.07	3.23	7.78	2.53
9/8/2009	10:05	MST-LV02-04	15	27	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	36.63	29.82	3.4	7.78	0.86
9/8/2009	10:02	MST-LV02-05	7	52	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	36.8	30.11	2.83	7.79	1.31
9/8/2009	9:05	MST-LV02-06	22	0	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	36.85	29.85	4.44	7.76	0.89
9/8/2009	9:00	MST-LV02-07	7	7	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	36.17	29.34	3.12	7.56	0.77
9/8/2009	8:29	MST-LV02-08	5	1	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	36.54	29.64	4.23	7.65	2.79
9/8/2009	8:54	MST-LV02-09	300	30	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	36.67	29.27	2.72	7.71	8.63
9/15/2009	10:10	MST-LV03-01	62	15	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	35.94	30.91	4.96	7.74	1.15
9/15/2009	9:55	MST-LV03-02	3	39	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	36.21	30.77	5.5	7.81	2.3
9/15/2009	10:19	MST-LV03-03	12	16	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	35.99	30.98	5.19	7.74	0.98
9/15/2009	9:34	MST-LV03-04	26	19	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	35.99	31.02	4.66	7.81	*
9/15/2009	9:52	MST-LV03-05	21	40	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	36.03	30.98	3.91	7.71	1.14
9/15/2009	8:55	MST-LV03-06	37	23	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	36.2	36.94	3.69	7.75	1.69
9/15/2009	9:12	MST-LV03-07	19	30	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	36.05	30.56	4.36	7.73	1.55
9/15/2009	8:28	MST-LV03-08	41	109	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	35.98	31.01	3.68	7.79	*
9/15/2009	8:32	MST-LV03-09	157	43	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	nd-E	36.03	31.04	3.96	7.82	3.22
9/28/2009	9:50	MST-LV04-01	12	5	113.4	0	0	(-)	(-)	0	0	5460	37.54	30.59	4.49	7.94	2.52
9/28/2009	9:20	MST-LV04-02	17	0	63	0	0	(-)	(-)	0	0	5230	36.99	30.57	5.98	7.9	2.93
9/28/2009	10:00	MST-LV04-03	17	0	209	1	0	(-)	(-)	0	0	3721	37.33	30.6	3.93	7.85	1.89
9/28/2009	9:30	MST-LV04-04	28	0	165.8	4	7	(-)	(-)	0	0	2837	37.05	30.71	5.27	7.91	3.52
9/28/2009	9:41	MST-LV04-05	34	11	215.1	3	2	(-)	(-)	0	0	3351	37.05	31.05	4.53	7.93	1.85
9/28/2009	9:00	MST-LV04-06	42	0	149.1	0	0	(-)	(-)	0	0	4179	36.82	30.56	4.85	7.9	2.39
9/28/2009	9:15	MST-LV04-07	60	0	208	2	0	(-)	(-)	0	0	4751	32.62	30	4.12	7.83	2.02
9/28/2009	8:35	MST-LV04-08	6	0	995.6	0	3	(-)	(-)	0	0	3642	37.05	30.63	3.48	7.78	5.25
9/28/2009	8:50	MST-LV04-09	26	28	115.4	0	0	(-)	(-)	0	0	4406	36.76	30.34	3.21	7.83	*

Date	Time	Sample ID Label	culturable Enteroccoci by MEI plate count CFU/100ml	Culturable Bacteroides by BBE plate count CFU/100ml	total Enterococci by entero1 qPCR GE/100ml	Human-specific Bacteroidales by BacHum-UCD qPCR GE/100ml	Human-specific Bacteroidales by HF183 qPCR GE/100ml	human-specific Methanobrevibacter smithi by nifH gene presence/absence endpoint PCR	human-specific enterococci by esp gene presence/absence endpoint PCR	human-specific adenovirus by hexon gene qPCR TSC/100ml	Dog-specific Bacteroidales by AOML DogBac qPCR TSC/100ml	Total Bacteroidales by AllBac - GenBac3 qPCR GE/100ml	salinity ppt	temp C	DO mg/l	pH	turbidity
10/27/2009	10:33	MST-LV05-01	39	0	6518.13	31	0	(-)	(-)	0	0	3643	36.94	28.06	5.11	7.83	3.14
10/27/2009	9:59	MST-LV05-02	2	18	9840.19	3	0	(+)	(+)	0	0	5034	36.96	26.95	5.84	7.8	4.65
10/27/2009	10:43	MST-LV05-03	484	19	11169.2	375	412	(-)	(-)	0	0	6247	36.54	28.39	5	7.81	2.98
10/27/2009	9:55	MST-LV05-04	8	5	5995.9	10	0	(+)	(-)	0	0	2898	37.11	28.3	4.16	7.78	2.12
10/27/2009	10:19	MST-LV05-05	71	4	3551.86	55	39	(-)	(-)	0	0	2025	35.99	28.34	4.37	7.71	0.77
10/27/2009	9:34	MST-LV05-06	21	5	4001.23	27	8	(-)	(-)	0	0	2777	37	28.29	3.85	7.78	1.71
10/27/2009	9:45	MST-LV05-07	32	38	3412.36	560	277	(-)	(-)	0	0	1777	32.62	28.41	2.55	7.55	1.41
10/27/2009	9:10	MST-LV05-08	528	5	6350.31	759	921	(-)	(+)	0	0	3708	35.99	27.94	5.47	7.53	3.37
10/27/2009	9:21	MST-LV05-09	840	8	4289.67	2010	1827	(+)	(+)	0	0	1881	36.64	27.94	4.76	7.76	2.67
11/12/2009	10:01	MST-LV06-01	2	1	6	1	0	(-)	(-)	0	0	123	*	*	*	*	4.17
11/12/2009	9:50	MST-LV06-02	0	5	7	1	0	(-)	(-)	0	0	460	*	*	*	*	4.49
11/12/2009	10:09	MST-LV06-03	6	3	8	1	0	(+)	(-)	0	0	391	*	*	*	*	2.68
11/12/2009	9:20	MST-LV06-04	1	0	9	1	0	(-)	(-)	0	0	232	*	*	*	*	2.61
11/12/2009	9:15	MST-LV06-05	22	23	64	0	3	(-)	(-)	0	0	604	*	*	*	*	2.74
11/12/2009	8:50	MST-LV06-06	3	1	10	0	0	(-)	(-)	0	169	289	36.59	25.06	4.61	7.67	2.99
11/12/2009	9:07	MST-LV06-07	4	0	7	0	0	(-)	(-)	0	0	150	35.67	24.94	3.54	7.62	2.59
11/12/2009	8:35	MST-LV06-08	16	3	11	12	5	(-)	(-)	0	128	1233	36.64	24.94	6.7	7.49	2.9
11/12/2009	8:45	MST-LV06-09	7	4	9	7	3	(-)	(-)	0	81	1044	36.69	24.86	7.23	7.67	2.9
11/23/2009	9:35	MST-LV07-01	5	8	8	0	0	(-)	(-)	0	140	114	36.81	25.8	4.79	7.89	*
11/23/2009	9:12	MST-LV07-02	1	7	3	0	0	(-)	(-)	0	270	764	36.92	25.47	5.93	7.92	*
11/23/2009	9:48	MST-LV07-03	6	11	12	0	0	(-)	(-)	0	0	165	36.78	25.92	3.96	7.91	*
11/23/2009	9:09	MST-LV07-04	4	4	2	0	0	(-)	(-)	0	0	106	36.78	25.59	4.21	7.8	*
11/23/2009	9:02	MST-LV07-05	1	20	26	0	0	(-)	(-)	0	0	139	36.39	25.91	4.13	7.75	*
11/23/2009	8:51	MST-LV07-06	0	0	37	0	0	(-)	(-)	0	0	100	36.23	25.85	4.41	7.71	*
11/23/2009	8:42	MST-LV07-07	6	1	10	0	0	(-)	(-)	0	0	105	36.11	25.95	3.11	7.67	*
11/23/2009	8:19	MST-LV07-08	260	13	122	0	3	(-)	(-)	0	1230	1021	36.69	25.66	4.7	7.51	*
11/23/2009	8:30	MST-LV07-09	3	10	9	0	0	(-)	(-)	0	810	382	36.67	25.42	2.82	7.69	*
12/8/2009	9:31	MST-LV08-01	2	1	1	0	2	(-)	(-)	0	0	4777	36.24	24.23	5.44	7.72	2.08
12/8/2009	8:47	MST-LV08-02	1	15	1	2	0	(-)	(-)	0	0	13490	36.22	24.05	6.14	7.76	5.7
12/8/2009	9:44	MST-LV08-03	6	0	1	0	0	(-)	(-)	0	0	6652	36.24	24.23	5.43	7.72	1.58
12/8/2009	8:43	MST-LV08-04	5	5	3	0	0	(-)	(-)	0	0	11460	35.84	24.06	4.27	7.61	2.06
12/8/2009	9:00	MST-LV08-05	3	2	3	0	0	(-)	(-)	0	0	6246	33.1	24.65	5	7.63	2.66
12/8/2009	8:36	MST-LV08-06	6	2	13	0	1	(-)	(-)	0	0	3958	34.41	24.09	4.08	7.48	0.93
12/8/2009	8:39	MST-LV08-07	10	0	1	0	0	(-)	(-)	0	0	1366	36.03	25.02	2.04	7.51	0.77
12/8/2009	8:20	MST-LV08-08	3	3	2	0	0	(-)	(-)	0	0	5660	35.55	23.65	6.74	6.65	2.92
12/8/2009	8:25	MST-LV08-09	3	4	4	0	3	(-)	(-)	0	0	9782	35.57	23.73	3.74	7.32	5.09

Date	Time	Sample ID Label	culturable Enteroccoci by MEI plate count CFU/100ml	Culturable Bacteroides by BBE plate count CFU/100ml	total Enterococci by entero1 qPCR GE/100ml	Human-specific Bacteroidales by BacHum-UCD qPCR GE/100ml	Human-specific Bacteroidales by HF183 qPCR GE/100ml	human-specific Methanobrevibacter smithi by nifH gene presence/absence endpoint PCR	human-specific enterococci by esp gene presence/absence endpoint PCR	human-specific adenovirus by hexon gene qPCR TSC/100ml	Dog-specific Bacteroidales by AOML DogBac qPCR TSC/100ml	Total Bacteroidales by AllBac - GenBac3 qPCR GE/100ml	salinity ppt	temp C	DO mg/l	pH	turbidity
12/22/2009	9:07	MST-LV09-01	2	0	15	2	0	(-)	(-)	0	0	22990	37.12	18.62	7.06	7.72	2.1
12/22/2009	9:03	MST-LV09-02	3	2	16	1	0	(+)	(-)	0	0	28270	37.16	17.54	7.61	7.71	2.5
12/22/2009	9:15	MST-LV09-03	7	5	21	0	0	(-)	(-)	0	5	49330	37.05	18.54	6.41	7.73	2.3
12/22/2009	8:42	MST-LV09-04	0	0	16	3	0	(-)	(-)	0	0	31230	37.06	18.02	6.19	7.69	1.5
12/22/2009	9:00	MST-LV09-05	1	0	9	2	0	(-)	(-)	0	0	42220	36.9	17.98	6.9	7.73	2.1
12/22/2009	8:33	MST-LV09-06	7	3	12	0	0	(-)	(-)	0	0	25460	36.63	18.91	5.83	7.65	1.6
12/22/2009	8:20	MST-LV09-07	5	4	18	0	0	(-)	(-)	0	0	23950	34.4	22.8	4.53	7.61	1.4
12/22/2009	8:03	MST-LV09-08	5	1	7	2	0	(-)	(-)	0	2	48430	36.7	16.51	8.42	7.62	2.1
12/22/2009	8:16	MST-LV09-09	2	1	4	2	0	(-)	(-)	0	7	41260	36.99	16.59	7.93	*	4.6
		MST-LV10 48hour Intensive (48+ hour Diurnal Study) - Jan 26-28, 2010, for sites 04 & 05															
1/26/2010	10:00	MST-LV10-04-1	3	0	33	1	0	(-)	(-)	0	1	784	35.88	20.27	3.62	7.75	3.7
1/26/2010	12:00	MST-LV10-04-2	3	2	51	0	0	(-)	(-)	0	2	991	35.89	20.27	4.14	7.77	3.8
1/26/2010	14:00	MST-LV10-04-3	1	0	57	0	0	(-)	(-)	0	0	1083	35.97	21	5.67	7.89	4.1
1/26/2010	16:00	MST-LV10-04-4	4	0	28	0	0	(-)	(-)	0	0	451	35.99	19.9	8.4	8	*
1/26/2010	18:00	MST-LV10-04-5	3	0	35	0	1	(-)	(-)	0	0	415	36.03	19.39	7.44	7.95	*
1/26/2010	20:00	MST-LV10-04-6	9	5	57	0	0	(-)	(-)	0	2	371	35.96	19.65	6.36	7.91	*
1/26/2010	22:00	MST-LV10-04-7	4	1	35	0	0	(-)	(-)	0	0	264	36.01	19.65	5.65	7.86	*
1/27/2010	0:00	MST-LV10-04-8	3	0	34	0	0	(-)	(-)	0	0	311	35.72	19.81	4.24	7.76	1.66
1/27/2010	2:00	MST-LV10-04-9	12	4	33	6	2	(-)	(-)	0	4	383	35.82	20.14	4.19	7.79	2.24
1/27/2010	4:00	MST-LV10-04-10	4	0	52	3	0	(-)	(-)	0	4	430	35.86	20.1	4.15	7.79	1.37
1/27/2010	6:00	MST-LV10-04-11	13	4	238	4	0	(-)	(-)	0	3	432	35.9	19.61	4.45	7.82	2.54
1/27/2010	8:00	MST-LV10-04-12	11	0	33	11	7	(-)	(-)	0	0	506	36.3	18.04	5.18	7.87	2.64
1/27/2010	10:00	MST-LV10-04-13	2	0	52	2	0	(-)	(-)	0	0	461	36.15	17.59	5.37	7.87	4.6
1/27/2010	12:00	MST-LV10-04-14	5	0	244	7	1	(-)	(-)	0	0	412	36.22	17.78	6.05	7.92	2.73
1/27/2010	14:00	MST-LV10-04-15	2	0	33	5	2	(-)	(-)	0	2	351	36.18	19.07	7.11	7.99	3.15
1/27/2010	16:00	MST-LV10-04-16	0	8	35	0	0	(-)	(-)	0	0	295	36.25	18.6	8.27	8.04	4.88
1/27/2010	18:00	MST-LV10-04-17	0	0	48	0	0	(-)	(-)	0	1	217	36.34	19.89	9.31	8.19	5.8
1/27/2010	20:00	MST-LV10-04-18	13	0	162	5	1	(-)	(-)	0	0	208	36.3	19.57	9.01	8.18	7
1/27/2010	22:00	MST-LV10-04-19	3	0	48	0	0	(-)	(-)	0	0	133	36.3	19.44	8.51	8.16	*
1/28/2010	0:00	MST-LV10-04-20	46	0	105	6	0	(-)	(-)	0	3	204	36.22	19.43	6.1	7.97	1.32
1/28/2010	2:00	MST-LV10-04-21	23	3	82	12	0	(-)	(-)	0	0	269	35.84	19.37	4.98	7.88	1.07
1/28/2010	4:00	MST-LV10-04-22	21	1	46	16	21	(-)	(-)	0	0	388	35.91	19.71	4.03	7.85	0.95
1/28/2010	6:00	MST-LV10-04-23	5	1	26	5	0	(-)	(-)	0	1	152	35.91	18.76	5.27	8.05	1.95
1/28/2010	8:00	MST-LV10-04-24	9	2	107	12	14	(+)	(-)	0	0	271	35.75	18.62	5.15	7.97	2.8
1/28/2010	10:00	MST-LV10-04-25	3	4	44	9	2	(-)	(-)	0	0	222	35.74	18.52	5.19	7.96	3.7
1/26/2010	10:00	MST-LV10-05-1	3	0	0	0	0	(-)	(-)	0	4	980	36.41	19.98	4.2	7.84	2.84
1/26/2010	12:00	MST-LV10-05-2	1	0	0	0	0	(-)	(-)	0	0	597	36.4	20.08	4.44	7.83	2.88
1/26/2010	14:00	MST-LV10-05-3	0	0	1	12	0	(-)	(-)	0	0	385	36.27	20.6	3.71	7.8	4.81
1/26/2010	16:00	MST-LV10-05-4	3	3	0	11	0	(-)	(-)	0	0	95	36.35	20.82	4.99	7.86	*
1/26/2010	18:00	MST-LV10-05-5	26	8	0	11	6	(-)	(-)	0	0	181	36.43	20	5.39	7.88	*
1/26/2010	20:00	MST-LV10-05-6	8	0	1	50	37	(+)	(+)	0	0	271	36.68	19.6	5.23	7.86	*
1/26/2010	22:00	MST-LV10-05-7	3	1	0	21	9	(-)	(-)	0	0	198	35.68	20.16	4.26	7.78	*
1/27/2010	0:00	MST-LV10-05-8	4	0	1	12	12	(-)	(-)	0	0	213	34.67	20.59	3.91	7.72	2.52

Date	Time	Sample ID Label	culturable Enteroccoci by MEI plate count CFU/100ml	Culturable Bacteroides by BBE plate count CFU/100ml	total Enterococci by entero1 qPCR GE/100ml	Human-specific Bacteroidales by BacHum-UCD qPCR GE/100ml	Human-specific Bacteroidales by HF183 qPCR GE/100ml	human-specific Methanobrevibacter smithi by nifH gene presence/absence endpoint PCR	human-specific enterococci by esp gene presence/absence endpoint PCR	human-specific adenovirus by hexon gene qPCR TSC/100ml	Dog-specific Bacteroidales by AOML DogBac qPCR TSC/100ml	Total Bacteroidales by AllBac - GenBac3 qPCR GE/100ml	salinity ppt	temp C	DO mg/l	pH	turbidity
1/27/2010	2:00	MST-LV10-05-9	2	0	0	4	0	(-)	(-)	0	0	148	36.09	20.67	3.87	7.74	2.31
1/27/2010	4:00	MST-LV10-05-10	4	0	0	7	3	(-)	(-)	0	11	198	36.46	20.68	3.74	7.74	2.54
1/27/2010	6:00	MST-LV10-05-11	1	3	0	8	4	(-)	(-)	0	8	142	36.61	20.58	4.27	7.78	2.12
1/27/2010	8:00	MST-LV10-05-12	7	8	1	8	0	(-)	(-)	0	5	228	36.63	18.98	4.97	7.87	2.94
1/27/2010	10:00	MST-LV10-05-13	9	0	6	37	19	(+)	(-)	0	1	154	36.87	18.07	5.45	7.89	3.06
1/27/2010	12:00	MST-LV10-05-14	0	0	1	4	0	(-)	(-)	0	4	234	37.08	17.86	5.81	7.95	2.99
1/27/2010	14:00	MST-LV10-05-15	36	0	1	10	3	(-)	(-)	0	2	160	36.72	18.19	6.1	7.94	2.97
1/27/2010	16:00	MST-LV10-05-16	2	0	2	29	21	(-)	(-)	0	6	170	36.66	18.98	6.64	7.96	3.863
1/27/2010	18:00	MST-LV10-05-17	0	3	1	6	0	(-)	(-)	0	4	164	36.71	18.88	6.81	7.97	4.28
1/27/2010	20:00	MST-LV10-05-18	0	2	4	13	8	(-)	(-)	0	2	111	36.72	19.3	7.79	8.05	5.04
1/27/2010	22:00	MST-LV10-05-19	1	0	1	16	22	(-)	(-)	0	0	155	36.45	19.51	6.15	7.95	1.72
1/28/2010	0:00	MST-LV10-05-20	12	0	1	16	19	(-)	(-)	0	0	115	35.58	19.68	5.77	7.89	1.17
1/28/2010	2:00	MST-LV10-05-21	10	0	3	8	1	(-)	(-)	0	4	117	35.03	19.92	5.02	7.84	1.34
1/28/2010	4:00	MST-LV10-05-22	32	0	6	8	2	(-)	(-)	0	4	71	35.89	19.86	4.61	7.84	1.1
1/28/2010	6:00	MST-LV10-05-23	7	0	5	18	7	(-)	(-)	0	0	201	36.43	19.72	5.01	7.91	1.21
1/28/2010	8:00	MST-LV10-05-24	4	3	7	27	30	(-)	(-)	0	6	143	36.5	19.64	5.25	7.93	1.84
1/28/2010	10:00	MST-LV10-05-25	1	3	3	5	1	(-)	(-)	0	0	109	36.53	19.48	5.14	7.95	1.7
2/9/2010	10:37	MST-LV11-01	11	0	1	2	0	(-)	(-)	0	2	435	34.9	20.18	6.28	7.7	3.74
2/9/2010	10:21	MST-LV11-02	7	1	0	2	0	(-)	(-)	0	0	214	35.21	19.61	7.21	7.81	6.24
2/9/2010	10:42	MST-LV11-03	15	0	0	2	0	(-)	(-)	0	6	605	34.81	20.33	6.33	7.72	1.51
2/9/2010	10:15	MST-LV11-04	6	0	1	0	0	(-)	(-)	0	4	240	35.21	19.53	7.33	7.75	4.94
2/9/2010	10:30	MST-LV11-05	3	2	3	0	2	(-)	(-)	0	0	91	32.03	20.53	6.93	7.58	0
2/9/2010	9:35	MST-LV11-06	10	1	0	0	0	(-)	(-)	0	2	166	35.27	20.12	5.83	7.92	1.94
2/9/2010	9:41	MST-LV11-07	2	0	0	1	0	(-)	(-)	0	0	65	31	20.62	4.1	7.45	0
2/9/2010	9:21	MST-LV11-08	13	2	86	1	3	(-)	(-)	0	0	517	35.23	19.29	6.17	7.37	2.89
2/9/2010	9:29	MST-LV11-09	25	3	141	2	7	(-)	(-)	0	0	319	34.23	19.23	7.02	7.59	1.86
3/2/2010	10:05	MST-LV12-01	7	0	0	0	0	(-)	(-)	0	0	171	36.11	18.72	7.23	8.06	4.45
3/2/2010	9:40	MST-LV12-02	2	3	0	1	1	(-)	(-)	0	0	195	36.23	18.83	6.93	7.94	6.36
3/2/2010	10:11	MST-LV12-03	13	0	0	0	0	(-)	(-)	0	0	98	35.99	18.99	7.14	8.01	3.85
3/2/2010	9:33	MST-LV12-04	4	0	4	0	1	(-)	(-)	0	0	216	36.16	18.96	6.84	8.01	3.95
3/2/2010	9:45	MST-LV12-05	1	2	94	0	0	(-)	(-)	0	0	137	34.48	19.88	5.53	7.79	0.92
3/2/2010	9:24	MST-LV12-06	3	1	0	0	0	(-)	(-)	0	0	126	36.25	19.78	4.74	7.91	0
3/2/2010	9:28	MST-LV12-07	13	0	3	0	0	(-)	(-)	0	0	160	26.76	20.34	4.7	7.64	1.6
3/2/2010	9:08	MST-LV12-08	3	7	62	2	2	(-)	(-)	0	3	242	36.23	18.29	6.28	8.02	1.94
3/2/2010	9:12	MST-LV12-09	4	1	273	0	1	(-)	(-)	0	16	389	34.91	18.37	6.56	7.95	0.99
3/17/2010	10:10	MST-LV13-01	12	2	20	2	1	(-)	(-)	0	5	24102	35.72	20.8	3.12	7.86	4
3/17/2010	9:42	MST-LV13-02	7	4	29	2	2	(-)	(-)	0	0	58104	35.96	19.84	5.36	7.86	4.96
3/17/2010	10:15	MST-LV13-03	15	3	38	8	2	(-)	(-)	0	0	70178	35.77	21.25	3.61	7.88	4.82
3/17/2010	9:40	MST-LV13-04	6	2	6	0	1	(-)	(-)	0	0	20654	35.84	21.05	4.8	7.78	2.84
3/17/2010	9:50	MST-LV13-05	3	4	17	0	0	(-)	(-)	0	0	19295	35.05	21.76	0.64	7.76	2.68
3/17/2010	9:21	MST-LV13-06	6	3	15	0	0	(-)	(-)	0	0	15195	35.74	22.34	3.4	7.56	1.79
3/17/2010	9:24	MST-LV13-07	11	1	3	0	0	(-)	(-)	0	3	5501	35.87	22.41	4.03	7.62	2.48
3/17/2010	9:10	MST-LV13-08	15	1	24	3	0	(-)	(-)	0	0	7814	35.81	20.66	3.64	7.51	4.37
3/17/2010	9:14	MST-LV13-09	16	1	33	0	0	(-)	(+)	0	2	10531	35.77	21.18	3.47	7.61	5.76

Date	Time	Sample ID Label	culturable Enteroccoci by MEI plate count CFU/100ml	Culturable Bacteroides by BBE plate count CFU/100ml	total Enterococci by entero1 qPCR GE/100ml	Human-specific Bacteroidales by BacHum-UCD qPCR GE/100ml	Human-specific Bacteroidales by HF183 qPCR GE/100ml	human-specific Methanobrevibacter smithi by nifH gene presence/absence endpoint PCR	human-specific enterococci by esp gene presence/absence endpoint PCR	human-specific adenovirus by hexon gene qPCR TSC/100ml	Dog-specific Bacteroidales by AOML DogBac qPCR TSC/100ml	Total Bacteroidales by AllBac - GenBac3 qPCR GE/100ml	salinity ppt	temp C	DO mg/l	pH	turbidity
3/30/2010	10:12	MST-LV14-01	6	3	0	0	0	(-)	(-)	0	0	15474	35.86	22.77	4.54	7.76	0.62
3/30/2010	9:58	MST-LV14-02	5	1	0	0	0	(-)	(-)	0	0	28839	36.26	20.17	6.04	7.85	2.08
3/30/2010	10:17	MST-LV14-03	16	3	0	0	0	(-)	(-)	0	0	23491	35.8	22.67	5.65	7.86	1.71
3/30/2010	9:56	MST-LV14-04	9	5	0	0	0	(-)	(-)	0	0	26277	35.91	22.26	5.43	7.79	1.11
3/30/2010	10:00	MST-LV14-05	4	4	2	0	0	(-)	(-)	0	2	19498	35.44	23.42	5.25	7.74	0.44
3/30/2010	9:32	MST-LV14-06	5	2	0	1	0	(-)	(-)	0	0	6362	36.27	23.12	5.01	7.68	0.3
3/30/2010	9:41	MST-LV14-07	2	2	2	0	0	(-)	(-)	0	2	12515	36.49	23.33	4.42	7.65	0
3/30/2010	9:14	MST-LV14-08	11	3	2	3	0	(-)	(-)	0	2	41581	36.16	21.64	3.9	7.63	2.76
3/30/2010	9:21	MST-LV14-09	24	3	6	0	0	(-)	(-)	0	6	19412	35.83	21.54	3.82	7.72	3.01
4/13/2010	10:00	MST-LV15-01	5	6	2	0	0	(-)	(-)	0	0	539	36.38	24.87	6.2	7.9	0.68
4/13/2010	9:45	MST-LV15-02	5	5	6	0	0	(+)	(-)	0	0	448	36.59	22.92	6.01	7.74	2.95
4/13/2010	10:15	MST-LV15-03	4	9	5	0	0	(-)	(-)	0	0	453	36.75	24.71	5.63	7.94	1.13
4/13/2010	9:40	MST-LV15-04	11	5	8	0	0	(-)	(-)	0	0	470	36.56	24.42	5.66	7.84	0.79
4/13/2010	9:50	MST-LV15-05	1	5	44	0	0	(-)	(-)	0	0	970	36.13	24.9	4.95	7.87	0.08
4/13/2010	9:20	MST-LV15-06	5	6	7	0	0	(-)	(-)	0	0	260	36.61	24.6	5.26	7.66	0
4/13/2010	9:28	MST-LV15-07	4	5	7	0	0	(-)	(-)	0	96	669	36.83	24.88	4.39	7.69	0.88
4/13/2010	9:08	MST-LV15-08	6	8	16	0	0	(-)	(-)	0	0	235	36.42	23.72	5.43	6.93	1.69
4/13/2010	9:15	MST-LV15-09	6	9	29	0	0	(-)	(-)	0	69	369	36.12	24.19	3.06	7.44	3.91
4/29/2010	10:35	MST-LV16-01	4	11	6	0	0	(-)	(-)	0	677	352	37.2	26.33	7.56	8.06	*
4/29/2010	10:15	MST-LV16-02	7	34	1	0	0	(-)	(-)	0	682	363	37.2	26.58	7.62	8.12	*
4/29/2010	10:40	MST-LV16-03	4	17	8	0	0	(-)	(-)	0	1014	565	37.3	26.43	7.36	8.08	*
4/29/2010	10:15	MST-LV16-04	0	14	6	0	0	(-)	(-)	0	463	355	37.2	26.58	7.62	8.12	*
4/29/2010	10:27	MST-LV16-05	0	9	5	0	0	(-)	(-)	0	353	228	36.23	26.56	7.35	8.05	*
4/29/2010	9:53	MST-LV16-06	7	10	1	0	0	(-)	(-)	0	640	240	36.91	26.58	6.29	8.03	*
4/29/2010	9:59	MST-LV16-07	23	1	19	0	0	(-)	(-)	0	723	377	36	26.03	6.25	7.76	*
4/29/2010	9:40	MST-LV16-08	3	5	3	0	0	(-)	(-)	0	812	437	37.27	26.6	4.65	7.99	*
4/29/2010	9:50	MST-LV16-09	4	11	1	0	0	(-)	(-)	0	811	477	37.01	26.51	5.64	7.99	*
5/11/2010	10:00	MST-LV17-01	3	15	3	0	0	(-)	(-)	0	957	682	38.03	27.79	5.33	8.13	3
5/11/2010	9:45	MST-LV17-02	1	0	4	0	0	(-)	(-)	0	404	438	38.15	26.15	5.51	7.96	4.04
5/11/2010	9:55	MST-LV17-03	4	1	3	0	0	(+)	(-)	0	1121	907	38.05	27.62	5.57	8.08	3.06
5/11/2010	9:45	MST-LV17-04	0	21	2	0	0	(-)	(-)	0	858	579	37.99	27.05	5.33	7.98	3.3
5/11/2010	9:50	MST-LV17-05	0	14	1	0	0	(+)	(-)	0	608	524	37.59	27.8	5.09	8.02	2.79
5/11/2010	9:30	MST-LV17-06	3	9	13	0	0	(-)	(-)	0	514	234	37.8	27.84	7.93	7.93	2.35
5/11/2010	9:35	MST-LV17-07	1	0	2	0	0	(-)	(-)	0	658	141	37.51	27.46	5.02	7.88	1.38
5/11/2010	9:20	MST-LV17-08	3	3	0	0	7	(+)	(+)	0	539	1583	37.86	27.51	nd-I	7.75	3.24
5/11/2010	9:23	MST-LV17-09	2	8	3	0	0	(-)	(-)	0	412	2107	37.84	27.66	nd-I	7.87	2.83

Date	Time	Sample ID Label	culturable Enteroccoci by MEI plate count CFU/100ml	Culturable Bacteroides by BBE plate count CFU/100ml	total Enterococci by entero1 qPCR GE/100ml	Human-specific Bacteroidales by BacHum-UCD qPCR GE/100ml	Human-specific Bacteroidales by HF183 qPCR GE/100ml	human-specific Methanobrevibacter smithi by nifH gene presence/absence endpoint PCR	human-specific enterococci by esp gene presence/absence endpoint PCR	human-specific adenovirus by hexon gene qPCR TSC/100ml	Dog-specific Bacteroidales by AOML DogBac qPCR TSC/100ml	Total Bacteroidales by AllBac - GenBac3 qPCR GE/100ml	salinity ppt	temp C	DO mg/l	pH	turbidity
5/25/2010	9:59	MST-LV18-01	0	7	3	0	0	(-)	(-)	0	633	215	37.32	28.84	6.09	8.25	2.5
5/25/2010	9:45	MST-LV18-02	0	13	15	0	0	(-)	(-)	0	685	358	37.25	28.18	53.04	8.25	3.02
5/25/2010	10:05	MST-LV18-03	0	5	6	0	0	(-)	(-)	0	588	203	37.01	28.87	5.8	8.26	2.5
5/25/2010	9:40	MST-LV18-04	0	5	1	0	0	(-)	(-)	0	673	199	37.05	28.96	6.03	8.25	1.7
5/25/2010	9:50	MST-LV18-05	0	5	11	0	0	(-)	(-)	0	1256	479	36.59	28.64	5.18	8.26	1.5
5/25/2010	9:30	MST-LV18-06	6	24	3	0	0	(-)	(-)	0	936	195	36.59	28.64	5.18	8.2	1.5
5/25/2010	9:31	MST-LV18-07	4	2	0	0	0	(-)	(-)	0	531	265	34.7	27.5	5.29	7.97	0.75
5/25/2010	9:17	MST-LV18-08	1	23	3	0	0	(-)	(-)	0	321	374	36.23	28.52	4.21	8.16	2.4
5/25/2010	9:23	MST-LV18-09	0	16	4	0	0	(-)	(-)	0	233	506	36.23	28.52	4.21	8.16	2.4
6/8/2010	9:52	MST-LV19-01	0	21	29	0	0	(-)	(-)	0	85	546	38.66	32.81	4.24	7.97	nd-l
6/8/2010	9:38	MST-LV19-02	6	14	17	0	0	(-)	(-)	0	0	350	38.56	32.57	2.9	7.91	nd-l
6/8/2010	9:45	MST-LV19-03	0	17	21	0	0	(-)	(-)	0	362	1071	38.63	32.78	4.04	7.96	nd-l
6/8/2010	9:38	MST-LV19-04	32	12	18	0	0	(-)	(-)	0	0	430	38.45	33.04	3.47	7.93	nd-l
6/8/2010	9:42	MST-LV19-05	0	9	8	0	2	(-)	(-)	0	0	1847	36.38	32.3	3.1	7.84	nd-l
6/8/2010	9:22	MST-LV19-06	1	23	8	0	0	(-)	(-)	0	0	257	38.35	32.63	3.64	8.03	nd-l
6/8/2010	9:29	MST-LV19-07	0	19	32	5	13	(+)	(-)	0	0	212	33.17	31.61	2.94	7.85	nd-l
6/8/2010	8:56	MST-LV19-08	60	42	26	0	4	(-)	(-)	0	0	330	38.13	32.56	2.95	8.09	nd-l
6/8/2010	9:09	MST-LV19-09	3	57	12	0	6	(-)	(-)	0	0	187	37.99	32.61	2.05	8.08	nd-l
6/23/2010	10:29	MST-LV20-01	3	10	2	0	0	(-)	(-)	0	37	342	38.57	30.45	4.75	8.17	1.6
6/23/2010	10:00	MST-LV20-02	1	6	4	0	2	(-)	(-)	0	0	238	38.57	29.64	5.07	8.02	2.3
6/23/2010	10:36	MST-LV20-03	2	3	0	0	0	(-)	(-)	0	0	227	38.6	30.49	4.8	8.19	2
6/23/2010	10:10	MST-LV20-04	0	8	3	0	0	(-)	(-)	0	0	160	38.66	30.25	5.15	8.08	1.9
6/23/2010	10:22	MST-LV20-05	1	11	2	0	1	(-)	(-)	0	0	756	38.24	30.63	3.64	8.08	1.6
6/23/2010	9:39	MST-LV20-06	1	5	1	0	0	(-)	(-)	0	0	193	38.27	30.03	4.4	8.11	1.1
6/23/2010	9:45	MST-LV20-07	7	23	11	0	0	(-)	(-)	0	0	287	36.94	28.96	5.14	7.99	0.85
6/23/2010	9:29	MST-LV20-08	116	13	31	3	2	(-)	(-)	0	8	673	38.43	29.35	2.76	2.93	1.5
6/23/2010	9:32	MST-LV20-09	4	9	12	4	0	(-)	(-)	0	11	622	38.09	29.03	2.65	7.93	2.4
7/7/2010	10:25	MST-LV21-01	3	19	4	0	0	(-)	(-)	0	91	320	nd-l	nd-l	nd-l	nd-l	nd-l
7/7/2010	10:01	MST-LV21-02	3	3	7	0	0	(-)	(-)	0	0	187	nd-l	nd-l	nd-l	nd-l	nd-l
7/7/2010	10:30	MST-LV21-03	6	3	2	0	0	(-)	(-)	0	48	222	nd-l	nd-l	nd-l	nd-l	nd-l
7/7/2010	10:00	MST-LV21-04	4	4	2	4	0	(-)	(-)	0	10	119	nd-l	nd-l	nd-l	nd-l	nd-l
7/7/2010	10:10	MST-LV21-05	1	6	1	0	0	(-)	(-)	0	0	6	nd-l	nd-l	nd-l	nd-l	nd-l
7/7/2010	9:45	MST-LV21-06	1	5	0	0	0	(-)	(-)	0	0	53	nd-l	nd-l	nd-l	nd-l	nd-l
7/7/2010	9:51	MST-LV21-07	1	3	2	0	0	(-)	(-)	0	0	63	nd-l	nd-l	nd-l	nd-l	nd-l
7/7/2010	9:36	MST-LV21-08	3	48	10	3	0	(-)	(-)	0	0	692	nd-l	nd-l	nd-l	nd-l	nd-l
7/7/2010	9:39	MST-LV21-09	1	5	3	6	0	(-)	(-)	0	0	150	nd-l	nd-l	nd-l	nd-l	nd-l

Date	Time	Sample ID Label	culturable Enteroccoci by MEI plate count CFU/100ml	Culturable Bacteroides by BBE plate count CFU/100ml	total Enterococci by entero1 qPCR GE/100ml	Human-specific Bacteroidales by BacHum-UCD qPCR GE/100ml	Human-specific Bacteroidales by HF183 qPCR GE/100ml	human-specific Methanobrevibacter smithi by nifH gene presence/absence endpoint PCR	human-specific enterococci by esp gene presence/absence endpoint PCR	human-specific adenovirus by hexon gene qPCR TSC/100ml	Dog-specific Bacteroidales by AOML DogBac qPCR TSC/100ml	Total Bacteroidales by AllBac - GenBac3 qPCR GE/100ml	salinity ppt	temp C	DO mg/l	pH	turbidity
MST-LV22 48hour Intensive 48+ hour Diurnal Study - July 19-22, 2010, for sites 04 & 05																	
7/19/2010	13:00	MST-LV22-04-1	3	0	17	0.0	0	(-)	(-)	0	0	429	*	*	*	*	*
7/19/2010	15:00	MST-LV22-04-2	2	10	6	0.5	0	(-)	(-)	0	0	228	36.9	29.14	4.21	8.05	0.0
7/19/2010	17:00	MST-LV22-04-3	3	5	4	0.0	0	(-)	(-)	0	0	141	36.99	30.66	8.27	8.33	0.0
7/19/2010	19:00	MST-LV22-04-4	1	3	1	0.0	0	(-)	(-)	0	0	140	36.91	32.07	9.26	8.42	0.0
7/19/2010	21:00	MST-LV22-04-5	6	6	6	5.7	0	(-)	(-)	0	10	338	36.89	32.11	9.05	8.48	0.0
7/19/2010	23:00	MST-LV22-04-6	5	19	6	0.0	0	(-)	(-)	0	0	818	36.79	31.66	7.72	8.41	0.0
nd-S	nd-S	MST-LV22-04-7	nd	nd	nd	nd	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S
nd-S	nd-S	MST-LV22-04-8	nd	nd	nd	nd	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S
7/20/2010	5:00	MST-LV22-04-9	11	37	17	0.0	0	(-)	(-)	0	0	637	37.04	30.62	5.56	8.33	0.0
7/20/2010	7:00	MST-LV22-04-10	10	29	6	0.0	0	(-)	(-)	0	0	472	37.03	29.69	4.55	8.26	0.0
7/20/2010	9:00	MST-LV22-04-11	3	12	28	0.0	0	(-)	(-)	0	0	345	37	29.07	4.15	8.20	0.0
7/20/2010	11:00	MST-LV22-04-12	12	10	36	0.0	0	(-)	(-)	0	0	282	37.07	28.79	3.48	8.17	0.0
7/20/2010	13:00	MST-LV22-04-13	0	8	38	0.0	0	(-)	(-)	0	0	522	36.96	28.66	3.26	8.06	0.0
7/20/2010	15:00	MST-LV22-04-14	0	22	34	0.0	0	(-)	(-)	0	0	363	36.95	29.31	3.80	8.09	0.4
7/20/2010	17:00	MST-LV22-04-15	1	10	26	0.4	0	(-)	(-)	0	0	821	36.84	29.84	6.47	8.17	0.0
7/20/2010	19:00	MST-LV22-04-16	1	49	31	0.0	0	(-)	(-)	0	0	412	36.8	31.46	8.14	8.39	0.0
7/20/2010	21:00	MST-LV22-04-17	2	27	76	3.5	0	(-)	(-)	0	516	821	36.89	31.83	7.74	8.47	0.4
7/20/2010	23:00	MST-LV22-04-18	13	15	62	0.0	0	(-)	(-)	0	193	480	36.72	30.97	5.44	8.41	0.0
7/21/2010	1:00	MST-LV22-04-19	10	11	34	0.0	0	(-)	(-)	0	41	266	36.77	30.83	5.29	8.36	0.0
7/21/2010	3:00	MST-LV22-04-20	4	8	42	0.0	0	(-)	(-)	0	59	287	36.8	30.64	4.82	8.33	0.0
7/21/2010	5:00	MST-LV22-04-21	8	22	45	0.0	0	(-)	(-)	0	67	404	36.85	29.94	4.18	8.33	0.0
7/21/2010	7:00	MST-LV22-04-22	16	24	30	0.0	0	(-)	(-)	0	32	370	36.92	28.78	3.57	8.24	0.0
7/21/2010	9:00	MST-LV22-04-23	10	4	42	0.0	0	(-)	(-)	0	24	391	36.9	28.87	3.09	8.23	0.0
7/21/2010	11:00	MST-LV22-04-24	19	6	47	0.0	0	(-)	(-)	0	70	403	36.84	28.62	2.84	8.17	0.0
7/21/2010	13:00	MST-LV22-04-25	1	4	48	0.0	0	(-)	(-)	0	15	384	36.84	29.63	3.21	8.15	0.0
7/21/2010	15:00	MST-LV22-04-26	2	1	90	0.0	0	(-)	(-)	0	14	350	36.74	30.01	4.41	8.13	0.2
7/21/2010	17:00	MST-LV22-04-27	1	10	65	0.0	0	(-)	(-)	0	0	316	36.64	30.24	5.79	8.20	1.0
7/21/2010	19:00	MST-LV22-04-28	1	14	35	0.0	0	(-)	(-)	0	0	121	36.75	32.03	6.43	8.44	1.9
7/21/2010	21:00	MST-LV22-04-29	14	18	23	0.0	0	(-)	(-)	0	0	141	36.87	31.72	4.94	8.43	2.0
7/21/2010	23:00	MST-LV22-04-30	2	5	42	0.0	0	(-)	(-)	0	0	361	36.88	31.26	3.72	8.38	0.7
7/22/2010	1:00	MST-LV22-04-31	1	6	50	0.0	0	(-)	(-)	0	0	287	36.8	30.84	3.19	8.33	1.1
7/22/2010	3:00	MST-LV22-04-32	0	11	39	0.0	0	(-)	(-)	0	0	213	36.79	30.54	2.91	8.31	0.4
7/22/2010	5:00	MST-LV22-04-33	3	20	28	0.0	0	(-)	(-)	0	0	328	36.8	29.72	2.38	8.29	0.6
7/22/2010	7:00	MST-LV22-04-34	5	12	52	0.0	0	(-)	(-)	0	0	197	36.83	28.76	2.29	8.22	0.6
7/19/2010	13:00	MST-LV22-05-1	0	0	18	0.0	0	(-)	(-)	0	0	309	37.04	28.85	2.76	8.11	0.0
7/19/2010	15:00	MST-LV22-05-2	0	6	31	0.0	0	(-)	(-)	0	0	285	36.85	29.36	2.89	8.09	0.0
7/19/2010	17:00	MST-LV22-05-3	1	5	32	0.0	0	(-)	(-)	0	0	281	36.93	30.09	3.94	8.15	0.0
7/19/2010	19:00	MST-LV22-05-4	1	3	38	0.0	0	(-)	(-)	0	0	185	37.02	31.29	7.57	8.37	0.0
7/19/2010	21:00	MST-LV22-05-5	3	9	33	0.0	0	(-)	(-)	0	0	170	37.05	31.97	8.82	8.47	0.0
7/19/2010	23:00	MST-LV22-05-6	0	19	42	0.0	0	(-)	(-)	0	0	196	37.07	32.07	8.96	8.5	0.0
nd-S	nd-S	MST-LV22-05-7	nd	nd	nd	nd	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S
nd-S	nd-S	MST-LV22-05-8	nd	nd	nd	nd	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S	nd-S

Date	Time	Sample ID Label	culturable Enteroccoci by MEI plate count CFU/100ml	Culturable Bacteroides by BBE plate count CFU/100ml	total Enterococci by entero1 qPCR GE/100ml	Human-specific Bacteroidales by BacHum-UCD qPCR GE/100ml	Human-specific Bacteroidales by HF183 qPCR GE/100ml	human-specific Methanobrevibacter smithi by nifH gene presence/absence endpoint PCR	human-specific enterococci by esp gene presence/absence endpoint PCR	human-specific adenovirus by hexon gene qPCR TSC/100ml	Dog-specific Bacteroidales by AOML DogBac qPCR TSC/100ml	Total Bacteroidales by AllBac - GenBac3 qPCR GE/100ml	salinity ppt	temp C	DO mg/l	pH	turbidity
7/20/2010	5:00	MST-LV22-05-9	3	32	18	0.0	0	(-)	(-)	0	0	513	37.11	31.25	6.58	8.47	0.2
7/20/2010	7:00	MST-LV22-05-10	3	22	28	0.0	4	(+)	(-)	0	0	414	37.14	29.81	4.72	8.35	0.0
7/20/2010	9:00	MST-LV22-05-11	5	12	15	0.0	0	(-)	(-)	0	0	423	37.12	29.3	3.88	8.28	0.5
7/20/2010	11:00	MST-LV22-05-12	12	7	7	0.0	0	(-)	(-)	0	0	384	37.13	29.17	3.66	8.25	0.0
7/20/2010	13:00	MST-LV22-05-13	0	18	7	0.0	0	(-)	(-)	0	0	330	37.1	29.51	3.14	8.24	0.0
7/20/2010	15:00	MST-LV22-05-14	0	9	12	0.0	0	(-)	(-)	0	0	183	37.1	29.96	3.98	8.26	0.0
7/20/2010	17:00	MST-LV22-05-15	1	32	48	0.1	0	(-)	(-)	0	0	343	36.86	30.43	4.18	8.23	0.0
7/20/2010	19:00	MST-LV22-05-16	2	19	35	0.0	0	(-)	(-)	0	0	283	36.93	30.7	5.62	8.28	0.0
7/20/2010	21:00	MST-LV22-05-17	0	5	10	0.0	0	(-)	(-)	0	0	205	36.71	31.52	7.97	8.43	0.0
7/20/2010	23:00	MST-LV22-05-18	5	12	15	0.0	0	(-)	(-)	0	0	296	36.81	31.66	8.24	8.48	0.0
7/21/2010	1:00	MST-LV22-05-19	3	4	10	0.0	0	(-)	(-)	0	0	168	36.72	31.26	7.43	8.43	0.0
7/21/2010	3:00	MST-LV22-05-20	15	16	13	0.0	0	(-)	(-)	0	40	378	36.87	30.87	5.9	8.48	0.0
7/21/2010	5:00	MST-LV22-05-21	8	11	20	0.0	0	(-)	(-)	0	87	416	36.88	30.26	4.97	8.43	0.0
7/21/2010	7:00	MST-LV22-05-22	7	15	16	3.9	0	(-)	(-)	0	2	304	36.87	29.44	3.99	8.34	0.0
7/21/2010	9:00	MST-LV22-05-23	7	10	22	0.0	0	(-)	(-)	0	42	257	36.84	28.89	4.08	8.31	0.0
7/21/2010	11:00	MST-LV22-05-24	8	10	7	0.0	0	(-)	(-)	0	0	386	36.81	29.14	3.83	8.3	0.0
7/21/2010	13:00	MST-LV22-05-25	10	0	3	0.0	0	(-)	(-)	0	15	149	36.8	29.4	3.54	8.29	0.5
7/21/2010	15:00	MST-LV22-05-26	2	1	11	0.0	0	(-)	(-)	0	9	197	36.72	30.15	3.89	8.29	0.0
7/21/2010	17:00	MST-LV22-05-27	0	12	32	0.1	0	(-)	(-)	0	0	462	36.7	30.28	6.54	8.28	1.3
7/21/2010	19:00	MST-LV22-05-28	0	12	26	0.0	0	(-)	(-)	0	0	315	36.63	30.78	6.46	8.29	0.8
7/21/2010	21:00	MST-LV22-05-29	8	18	114	0.0	0	(-)	(-)	0	0	372	36.67	31.24	8.23	8.47	1.1
7/21/2010	23:00	MST-LV22-05-30	15	4	8	0.0	0	(-)	(-)	0	0	557	36.68	31.21	7.9	8.46	0.7
7/22/2010	1:00	MST-LV22-05-31	4	9	10	3.7	0	(-)	(-)	0	0	385	36.67	31.05	7.42	8.44	0.7
7/22/2010	3:00	MST-LV22-05-32	0	12	14	0.0	0	(-)	(-)	0	0	306	36.8	30.89	5.53	8.45	0.9
7/22/2010	5:00	MST-LV22-05-33	2	23	22	0.0	0	(-)	(-)	0	0	406	36.81	30.08	4.85	8.43	1.3
7/22/2010	7:00	MST-LV22-05-34	4	6	13	0.0	0	(-)	(-)	0	0	181	36.77	29.43	4.03	8.36	0.4
7/27/2010	10:36	MST-LV23-01	2	7	15	0	0	(-)	(-)	0	20	330	36.67	31.46	nd-l	8.11	1.55
7/27/2010	10:20	MST-LV23-02	1	7	25	0	0	(-)	(-)	0	0	150	36.88	30.82	nd-l	8.21	2.54
7/27/2010	10:40	MST-LV23-03	1	6	14	0	0	(-)	(-)	0	82	273	36.5	31.44	nd-l	8.1	1.14
7/27/2010	10:01	MST-LV23-04	6	2	34	0	0	(-)	(-)	0	0	85	36.7	31.51	nd-l	8.18	2.21
7/27/2010	10:10	MST-LV23-05	4	12	21	5	0	(-)	(-)	0	0	384	35.82	30.95	nd-l	8.06	0.41
7/27/2010	9:39	MST-LV23-06	17	3	4685	0	0	(-)	(-)	0	6	312	36.4	31.04	nd-l	8.18	1.62
7/27/2010	9:50	MST-LV23-07	5	7	84	0	0	(-)	(-)	0	0	704	32.37	31.22	nd-l	7.97	0.32
7/27/2010	9:30	MST-LV23-08	3	2	17	0	0	(-)	(-)	0	0	67	36.61	30.49	nd-l	8.16	2.06
7/27/2010	9:35	MST-LV23-09	3	4	30	0	0	(-)	(-)	0	0	336	36.3	31.6	nd-l	8.14	2.07

Date	Time	Sample ID Label	culturable Enteroccoci by MEI plate count CFU/100ml	Culturable Bacteroides by BBE plate count CFU/100ml	total Enterococci by entero1 qPCR GE/100ml	Human-specific Bacteroidales by BacHum-UCD qPCR GE/100ml	Human-specific Bacteroidales by HF183 qPCR GE/100ml	human-specific Methanobrevibacter smithi by nifH gene presence/absence endpoint PCR	human-specific enterococci by esp gene presence/absence endpoint PCR	human-specific adenovirus by hexon gene qPCR TSC/100ml	Dog-specific Bacteroidales by AOML DogBac qPCR TSC/100ml	Total Bacteroidales by AllBac - GenBac3 qPCR GE/100ml	salinity ppt	temp C	DO mg/l	pH	turbidity
8/24/2010	11:10	MST-LV24-01	14	1	24	1	0	(+)	(-)	0	0	446	35.1	30.06	3.01	7.86	2.06
8/24/2010	10:30	MST-LV24-02	0	0	9	0	0	(-)	(-)	0	0	664	35.07	29.33	5.98	8.03	4.28
8/24/2010	11:20	MST-LV24-03	34	3	63	0	0	(+)	(-)	0	0	473	35.12	30.01	2.9	7.85	1.84
8/24/2010	10:25	MST-LV24-04	7	0	15	0	1	(+)	(-)	0	0	1300	35.02	29.35	4.19	7.96	4.69
8/24/2010	10:55	MST-LV24-05	12	3	51	0	0	(-)	(-)	0	0	146	34.25	30.38	4.55	7.98	1.65
8/24/2010	9:55	MST-LV24-06	3	1	230	0	0	(+)	(-)	0	0	424	34.5	30.09	3.92	7.96	1.41
8/24/2010	10:05	MST-LV24-07	37	5	19	0	5	(+)	(-)	0	0	774	33.98	29.14	2.43	7.92	0.93
8/24/2010	9:25	MST-LV24-08	17	2	34	1	3	(-)	(-)	0	0	587	34.26	29.68	3.67	7.93	3.17
8/24/2010	9:40	MST-LV24-09	87	32	99	1	9	(+)	(-)	0	0	1262	34.19	30.02	2.83	7.87	2.31
9/4/2010	19:17	MST-LV25-01	2	0	26	0	0	(+)	(-)	0	1	750	34.93	31.95	7.68	8.23	12.8
9/4/2010	18:50	MST-LV25-02	3	0	40	1	0	(-)	(-)	0	0	506	34.67	32.01	8.96	8.35	3.84
9/4/2010	19:25	MST-LV25-03	2	1	89	0	0	(-)	(-)	0	4	735	33.35	31.1	4.4	7.96	3.1
9/4/2010	18:45	MST-LV25-04	9	6	44	0	0	(+)	(-)	0	0	773	34.03	32.46	9.24	8.34	2.87
9/4/2010	19:05	MST-LV25-05	13	6	1301	0	0	(+)	(-)	0	0	3202	27.12	30.35	4.79	7.81	4.83
9/4/2010	18:20	MST-LV25-06	12	24	144	0	0	(-)	(-)	0	0	1244	34.73	32.29	8.78	8.35	3.17
9/4/2010	18:30	MST-LV25-07	8	23	48	0	0	(-)	(-)	0	0	1463	24.26	29.13	4.1	7.63	1.07
9/4/2010	18:00	MST-LV25-08	22	12	58	4	10	(-)	(-)	0	0	7983	33.05	32.25	6.65	8.25	7.02
9/4/2010	18:10	MST-LV25-09	102	12	224	8	16	(+)	(+)	0	1	22839	33.19	32.22	5.46	8.13	2.83

* RED value: = Value reported is less than the laboratory Method Detection Limit (MDL).
* nd-E = Failed QC check for extraction- DNA extraction recovery too low, value could not be determined
* nd-S = Value could not be determined - ISCO autosampler failure, sample not collected
* nd-I = Value could not be determined - physical measurements not taken - field instruments not available
* = * pink highlight indicates values we consider "substantially elevated above background" for that marker, even though there are no regulatory standards for that marker
* = * red highlight indicates value in exceedance of regulatory standards for single-grab sample
* = * yellow highlight indicates missing value or value still pending
* = * blue highlight indicates anomolously low salinity value for that date's data set, suggesting fresh-water input at this site - note that all such lower salinity reading always seem to occur at either site 5 or site 7

Table 2: Frequency of Exceedances and Elevations for Fecal Indicators and Alternative Source Tracking Markers

Canal/site	Events (Exceedance or elevation)	Exposure Limit Exceedance of total Enterococci by mEI plate culture	Exposure Limit Exceedance of total Enterococci by qPCR	Substantial Elevation of Bacteroidales by BBE plate culture	Substantial Elevation of total general Bacteroidales by GenBac3 qPCR	Substatial Elevation of Human Specific Bacteroidales by BacHum-UCD qPCR	Substantial Elevation of Human-Specific Bacteroidales by HF183 qPCR	Substantial Elevation of Dog-Specific Bacteroides by AOML DogBac qPCR
91st St. Septic control canal	# of observations	46	42	46	42	42	42	42
	# of events	7	8	1	19	2	2	9
	% events	15.21%	19.04%	2.17%	45.23%	4.76%	4.76%	21.43%
97th St. Remediated canal	# of observations	46	42	46	41	42	42	42
	# of events	0	7	0	15	1	1	7
	% events	0%	16.67%	0%	36.56%	2.38%	2.38%	16.67%
100th St. Remediated canal (storm-water outfall)	# of observations	46	42	46	42	42	42	42
	# of events	0	6	0	16	0	0	9
	% events	0%	14.28%	0%	38.10%	0%	0%	21.43%
112th St. Remediated canal	# of observations	46	42	46	42	42	42	42
	# of events	1	4	0	15	1	1	8
	% events	2.17%	9.52%	0%	36.56%	2.38%	2.38%	19.05%
OffShore control site	# of observations	23	21	23	21	21	21	21
	# of events	0	1	0	7	0	0	4
	% events	0%	4.76%	0%	33.33%	0%	0%	19.05%

"Exceedance": >104/100mL, "Substantial Elevation": > 100/100 mL (Except for General Bacteroidales: > 1000/100mL)

Table 3: Frequency of Detecton of qPCR source tracking markers

Canal/site	Events – frequency of detection: qPCR detect = values > 5/100mL; endpoint PCR detect = values of "+" for presence/absence	Detection of total enterococci by entero1 qPCR	Detection of total Bacteroidales by AllBac – GenBac3 qPCR	Detection of Human Specific Bacteroidales by BacHum-UCD qPCR	Detection of Human Specific Bacteroidales by HF183 qPCR	Detection of Human Specific Methanobrevibacter smithii by Msmith nifH qPCR	Detection of Human Specific enterococci by esp gene qPCR	Detection of Human Specific Adenovirus by JVTX hexon gene qPCR	Detection of Dog Specific Bacteroides by AOML DogBac qPCR
91st St. Septic control canal	# of observations	42	42	42	42	42	42	42	42
	# of detections	31	42	6	8	4	5	0	17
	% detections	73.81%	100%	14.28%	19.05%	9.52%	11.90%	0%	40.48%
97th St. Remediated canal	# of observations	42	41	42	42	42	42	42	42
	# of detections	26	41	2	3	3	0	0	9
	% detections	61.90%	100%	4.76%	7.14%	7.14%	0%	0%	21.43%
100th St. Remediated canal (storm-water outfall)	# of observations	42	42	42	42	42	42	42	42
	# of detections	26	42	2	2	5	0	0	7
	% detections	61.90%	100%	4.76%	4.76%	11.90%	0%	0%	16.67%
112th St. Remediated canal	# of observations	42	42	42	42	42	42	42	42
	# of detections	25	42	3	1	5	0	0	16
	% detections	59.52%	100%	7.14%	2.38%	11.90%	0%	0%	38.09%
OffShore control site	# of observations	21	21	21	21	21	21	21	21
	# of detections	13	21	0	0	3	1	0	4
	% detections	61.90%	100%	0%	0%	7.14%	4.76%	0%	19.05%

Table 4: Frequency of detection of qPCR source tracking markers for 48hour intensive diurnal studies

100th St. Canal Sampling by Season	Events – frequency of detection: qPCR detect = values > 5/100mL; endpoint PCR detect = values of "+" for presence/absence	Detection of total enterococci by entero1 qPCR	Detection of total Bacteroidales by AllBac – GenBac3 qPCR	Detection of Human Specific Bacteroidales by BacHum-UCD qPCR	Detection of Human Specific Bacteroidales by HF183 qPCR	Detection of Human Specific Methanobrevibacter smithii by Msmith nifH qPCR	Detection of Human Specific enterococci by esp gene qPCR	Detection of Human Specific Adenovirus by JVTX hexon gene qPCR	Detection of Dog Specific Bacteroides by AOML DogBac qPCR
Winter Canal Total	# of observations	50	50	50	50	50	50	50	50
	# of detections	28	50	28	14	3	1	0	4
	% detections	56%	100%	56%	28%	6%	8%	0%	8%
Winter Canal Head	# of observations	25	25	25	25	25	25	25	25
	# of detections	3	25	20	11	2	1	0	4
	% detections	12%	100%	80%	44%	8%	4%	0%	16%
Winter Canal Mouth	# of observations	25	25	25	25	25	25	25	25
	# of detections	25	25	8	3	1	0	0	0
	% detections	100%	100%	32%	12%	4%	0%	0%	0%
Summer Canal Total	# of observations	64	64	64	64	64	64	64	64
	# of detections	61	64	1	0	0	0	0	16
	% detections	95.31%	100%	3.12%	0%	0%	0%	0%	25%
Summer Canal Head	# of observations	32	32	32	32	32	32	32	32
	# of detections	31	32	0	0	0	0	0	5
	% detections	99.85%	100%	0%	0%	0%	0%	0%	15.62%
Summer Canal Mouth	# of observations	32	32	32	32	32	32	32	32
	# of detections	30	32	1	0	0	0	0	11
	% detections	93.75%	100%	3.12%	0%	0%	0%	0%	34.37%

FIGURES

Figure 1b: Sampling Sites for the Little Venice Water Quality Monitoring Project and the Molecular Microbial Source Tracking Study. Site 2 is an offshore reference site. The 97th, 100th, and 112 Street homes have been connected to the new sewer system (i.e. the remediated canals), while the 91st St. homes (i.e. septic control canal) are still on individual septic field systems. There is a stormwater discharge culvert at the head of the 100th St. canal (marked in red) that drains the Marathon Airport area from the other side of US Highway 1 into the 100th St. canal – this canal was the site for the 48hour intensive diurnal samplings.

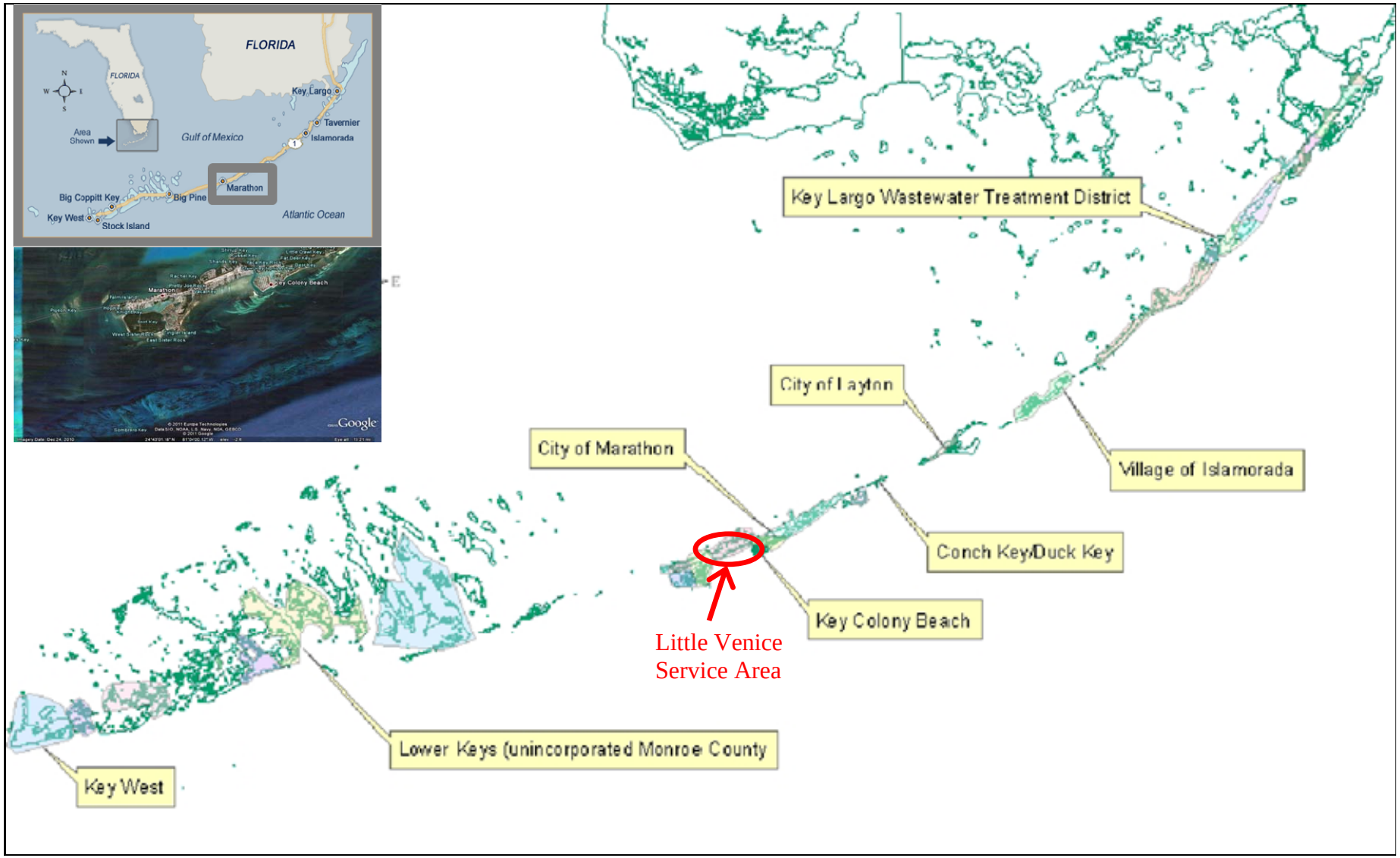

Figure 2: From the Monroe County Sanitary Wastewater Master Plan showing location of City of Marathon wastewater treatment plant. The location of the Little Venice Service Area is indicated by the the red circle. Operation of the Little Venice Sanitary Service Area has been transferred from the Florida Keys Aquaduct Authority to the City of Marathon, Florida.

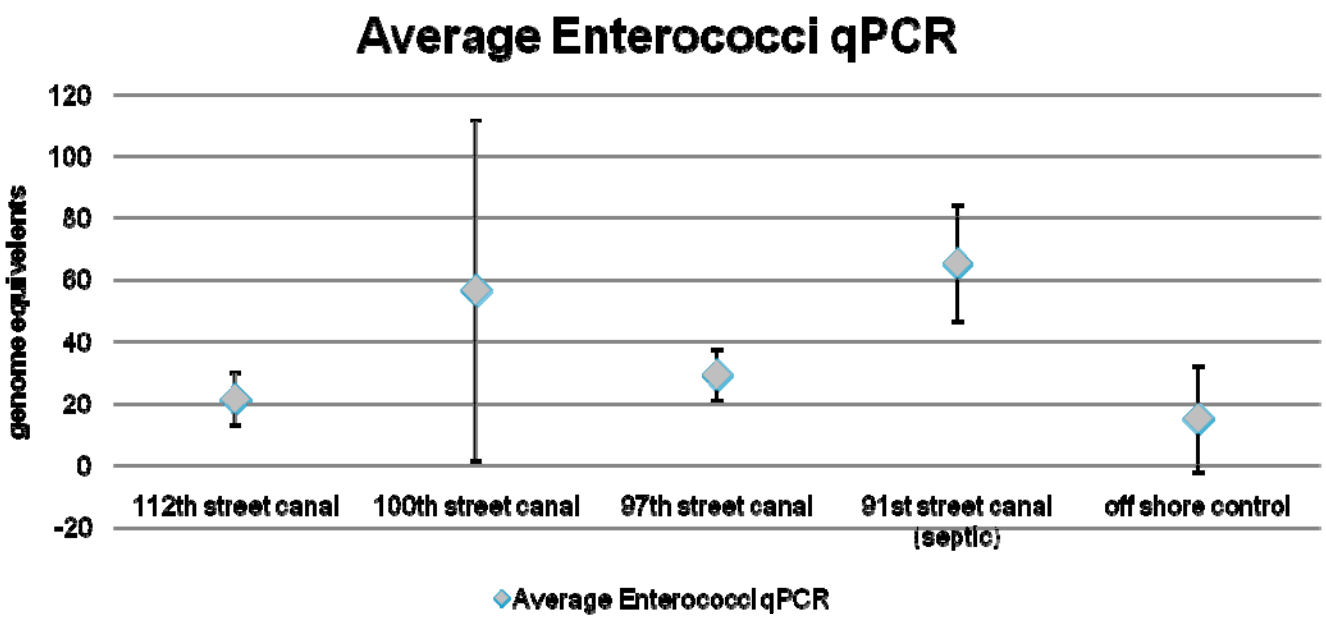

Figure 3: Overall averages of entero1 qPCR assay values for semi-monthly sampling by canal for total Enterococci abundance over entire duration of the study. Note the two sewered canals at 112th street and 97th street showed significant decrease in average enterococci abundance as compared to septic canal. The 100th Street canal with the stormwater discharge outfall showed average total Enterococci abundance intermediate to that of the septic canal and also showed the greatest variation in total Enterococci abundance, perhaps reflective of the differing hydrology of this canal.

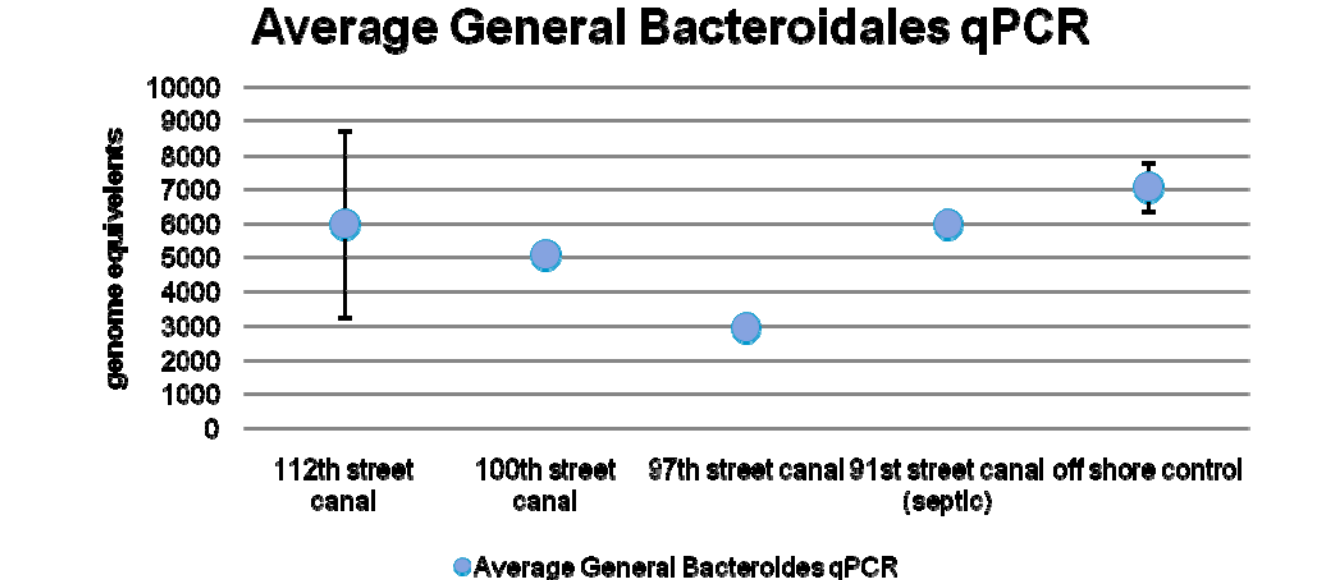

Figure 4: Overall average of the general Bacteroidales qPCR assay values for semi-monthly sampling by canal over entire duration of the study. The total Bacteroidales were ubiquitous and of relatively high abundance for all sample sites, including the offshore control site for most time points.

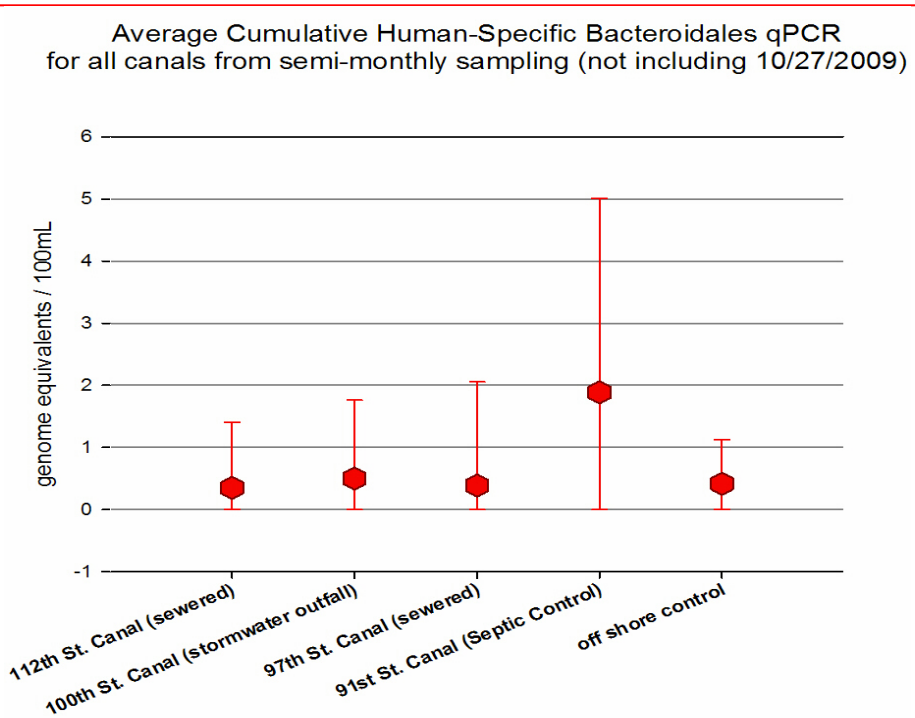

Figure 5: Overall average of cumulative human-specific Bacteroidales qPCR assay values (HF183 and BacHum-UCD markers combined) by canal for semi-monthly sampling over entire duration of the study (lower error bars to minimum value, upper error bars to 1st standard deviation). Values displayed do not include the extremely elevated outlier values of 10/27/2009. The septic control canal showed the greatest abundance and greatest variation for human Bacteroidales marker among the canals tested.

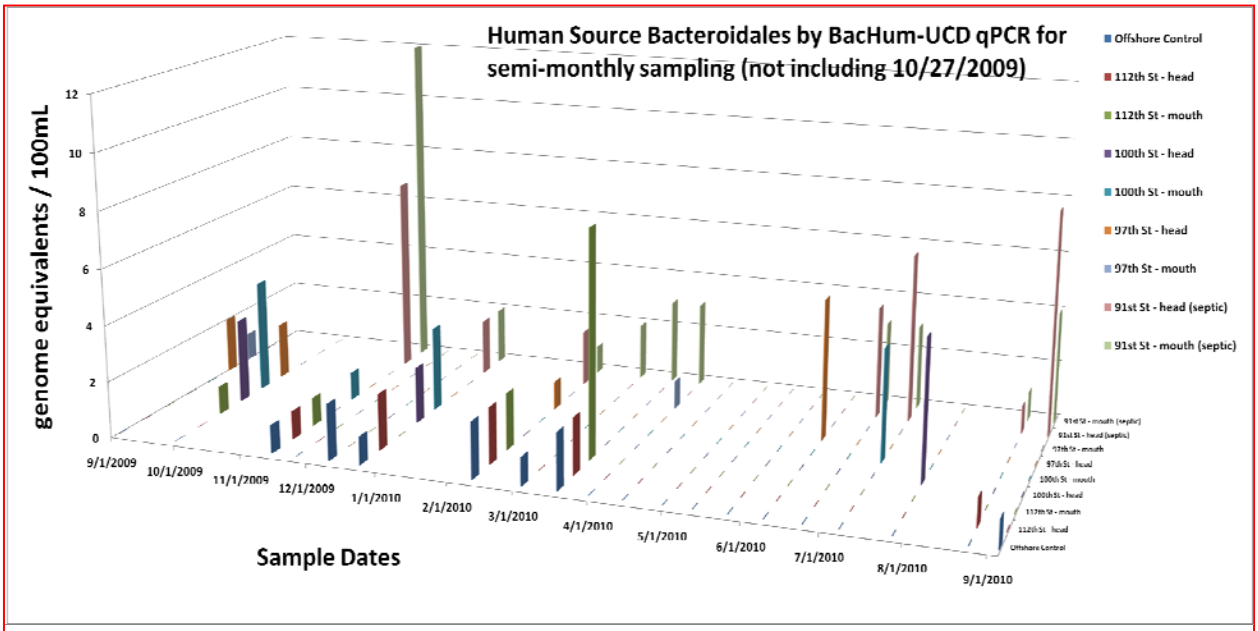

Figure 6: Human-specific Bacteroidales as measured by BacHum-UCD marker by canal head vs mouth for semi-monthly sampling over entire duration of the study. Offshore control shown in front and 91st St (septic) canal shown at back. Values displayed do not include the extremely elevated outlier values of 10/27/2009. The 91st St. septic control canal showed the greatest abundance and greatest frequency for human Bacteroidales marker among the canals tested.

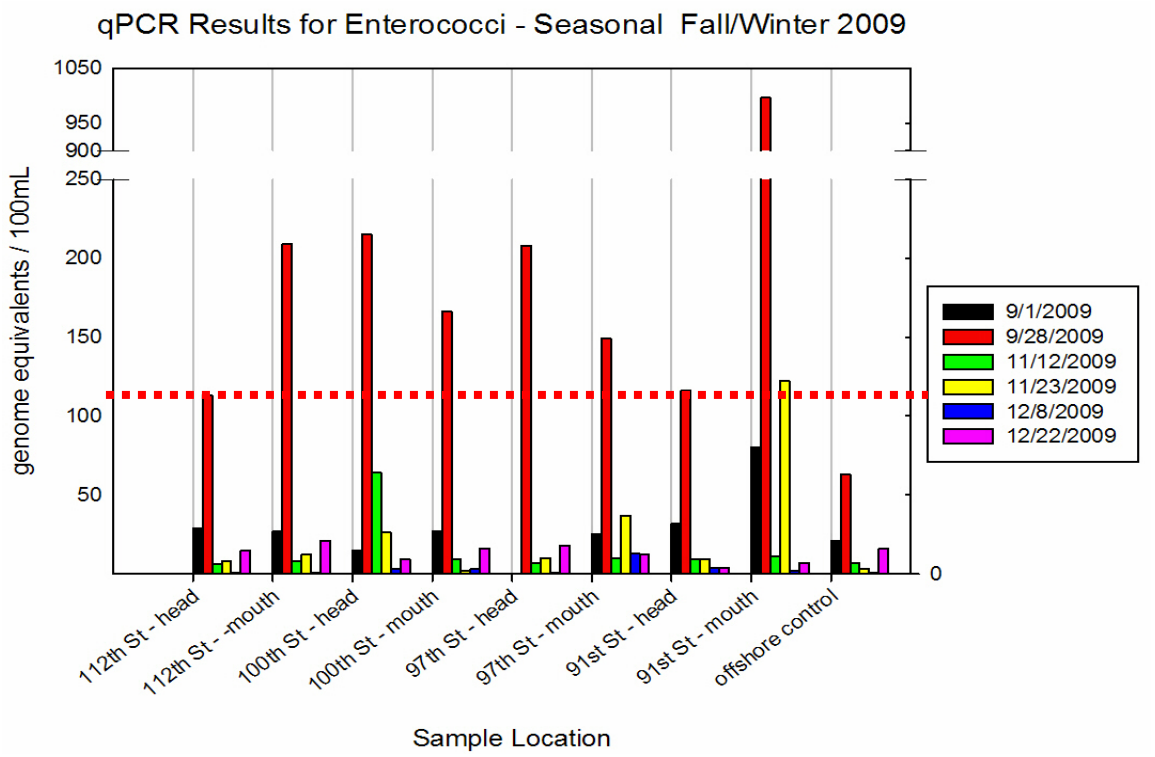

Figure 7: Enterococci abundance as measured by entero1 qPCR assay for Fall/Winter season of 2009 (Sept-Dec 2009). The 91st St. septic control canal showed the greatest abundance for most sample dates and greatest frequency for exceedence of regulatory limits (104 cells/100mL for single-grab samples – shown by red dotted line). Note that for date of 9/28/2009, all sites except the offshore control exceeded regulatory limits**.**

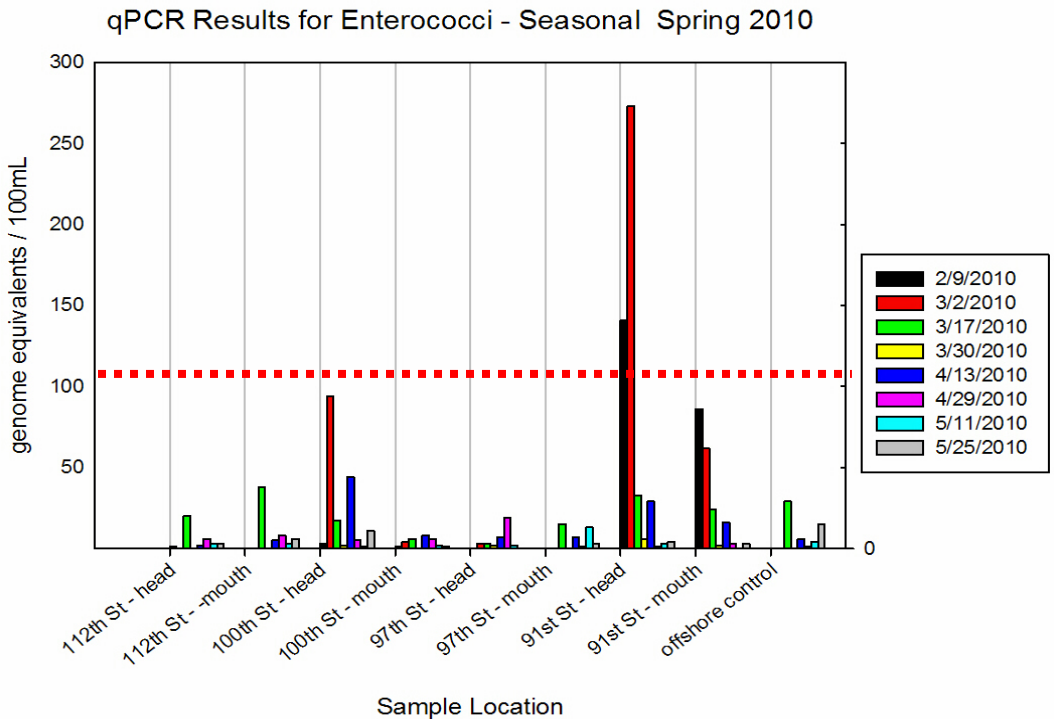

Figure 8: Enterococci abundance as measured by entero1 qPCR assay for the Spring season of 2010 (Feb-May 2009), including the start of the rainy season. The 91st St. septic control canal showed the greatest abundance for most sample dates and greatest frequency for exceedence of regulatory limits (104 cells/100mL for single-grab samples – shown by dotted red line). The head of the 100th St canal with the stormwater discharge also showed elevations of enterococci. For both the 91st St septic canal and 100th St. stormwater canal, levels were significantly higher at the heads than at the mouths of the canals.

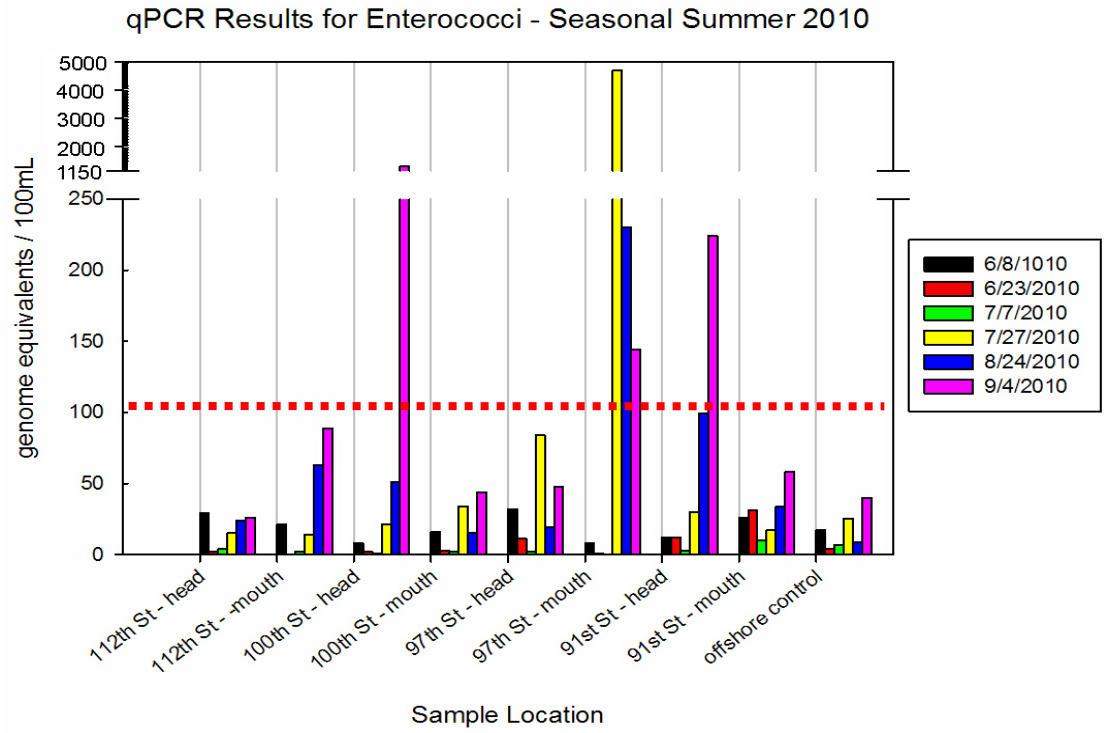

Figure 9: Enterococci abundance as measured by entero1 qPCR assay for the Summer "wet" season of 2010 (June-August 2010). The 91st St. septic control canal continued to show exceedance of regulatory limits (104 cells/100mL for single-grab samples as shown by dotted red line). However, the head of the 100th St canal with the stormwater discharge also showed some high exceedance, although levels for this canal were extremely variable during the season. In addition, the 97th St. canal showed some high exceedances for three dates (including one extremely high level on 7/27/2010), but only at the mouth. For both the 91st St septic canal and 100th St. stormwater canal, levels were significantly higher at the heads than at the mouths of the canals.

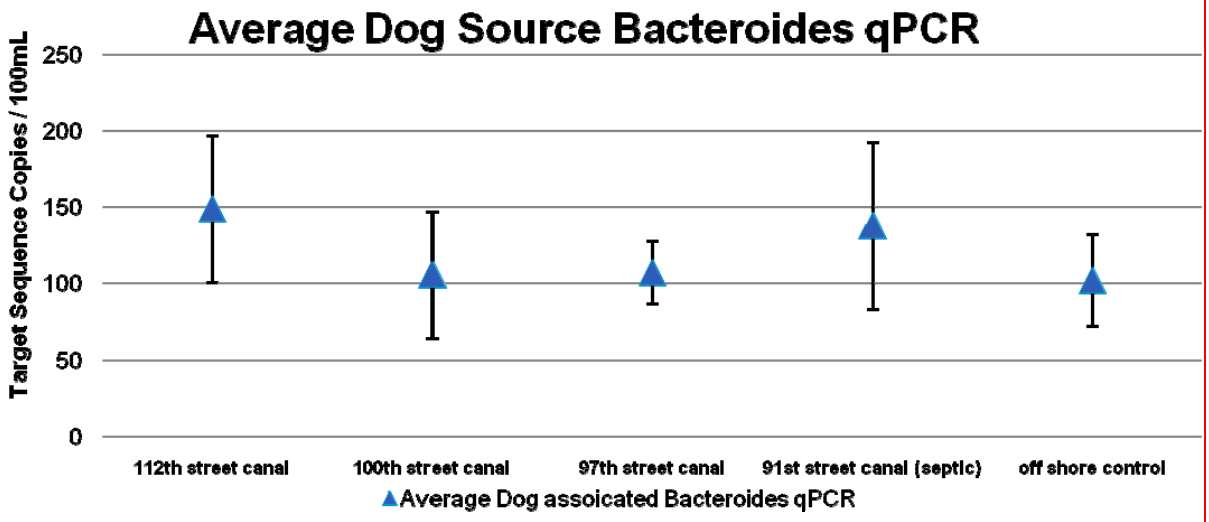

Figure 10: Overall average of cumulative dog-specific Bacteroides qPCR assay values (AOML DogBact marker) by canal for semi-monthly sampling over entire duration of the study. Moderate to high but variable levels of dog associated marker were found in all canals, with no significance difference between canals for cumulative average. The temporal and spatial pattern of dog-source fecal marker differs from the patterns of other source tracking markers and may be more reflective of local surface runoff.

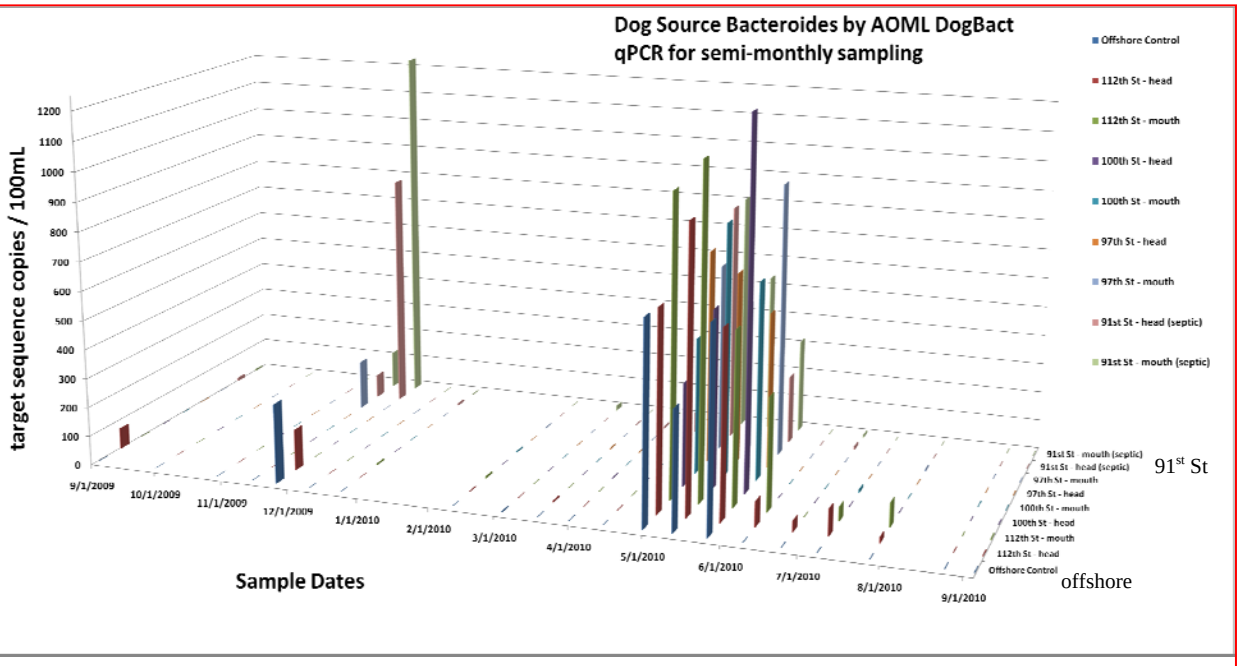

Figure 11: Dog-specific Bacteroides as measured by AOML DogBact qPCR marker by canal head vs mouth for semi-monthly sampling over entire duration of the study. Offshore control is shown in front and 91st St (septic) canal shown at back. All canals showed periodic but variable elevations. High levels of DogBact marker did not correlate to elevations of other source tracking markers. The head of the 112st St. canal showed the greatest frequency of elevations for dog-associated Bacteriodes. A one-month period from April 29 to May 25, 2010, near the beginning of the "wet season" showed high but variable levels of DogBact marker in all sample sites, including offshore.

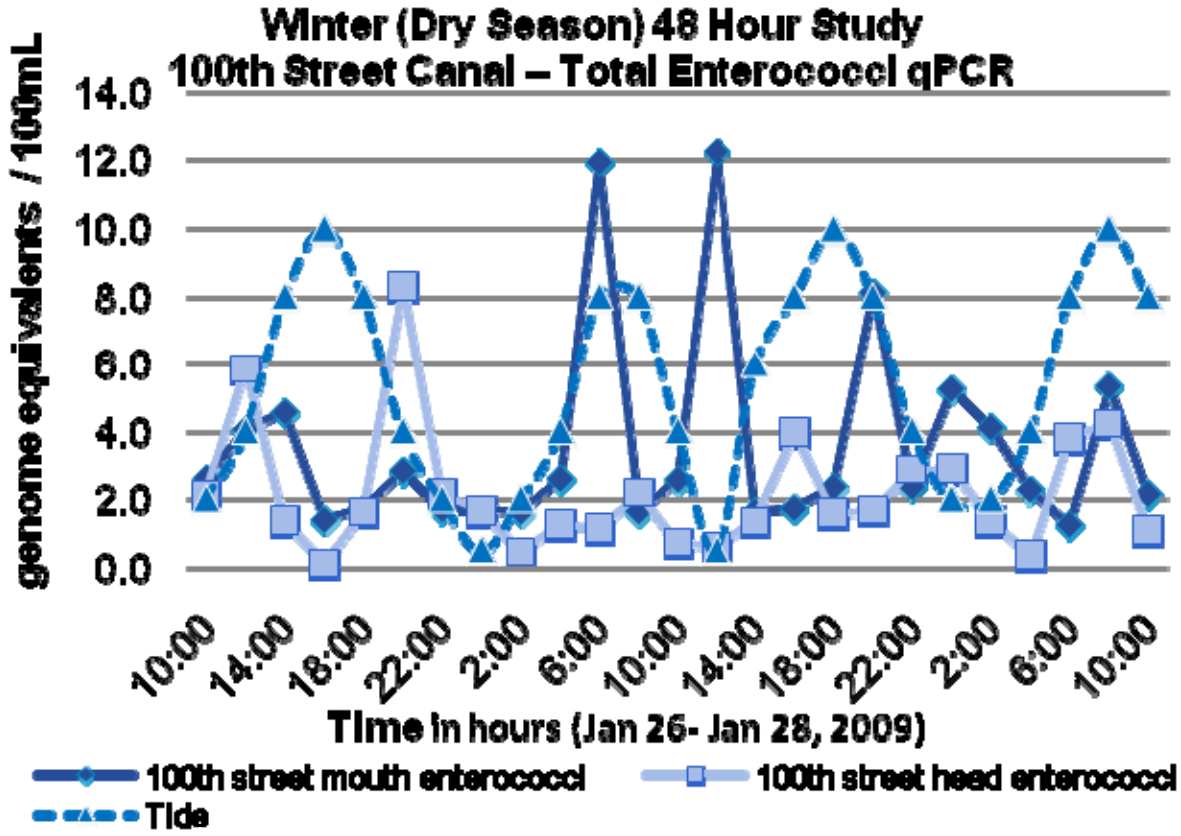

Figure 12: fluctuations of total enterococci over time at head and mouth of 100th Street Canal during the Winter (dry season) 48 hour diurnal study (Jan 26-28, 2009), as measured every 2 hours by the entero1 qPCR marker.

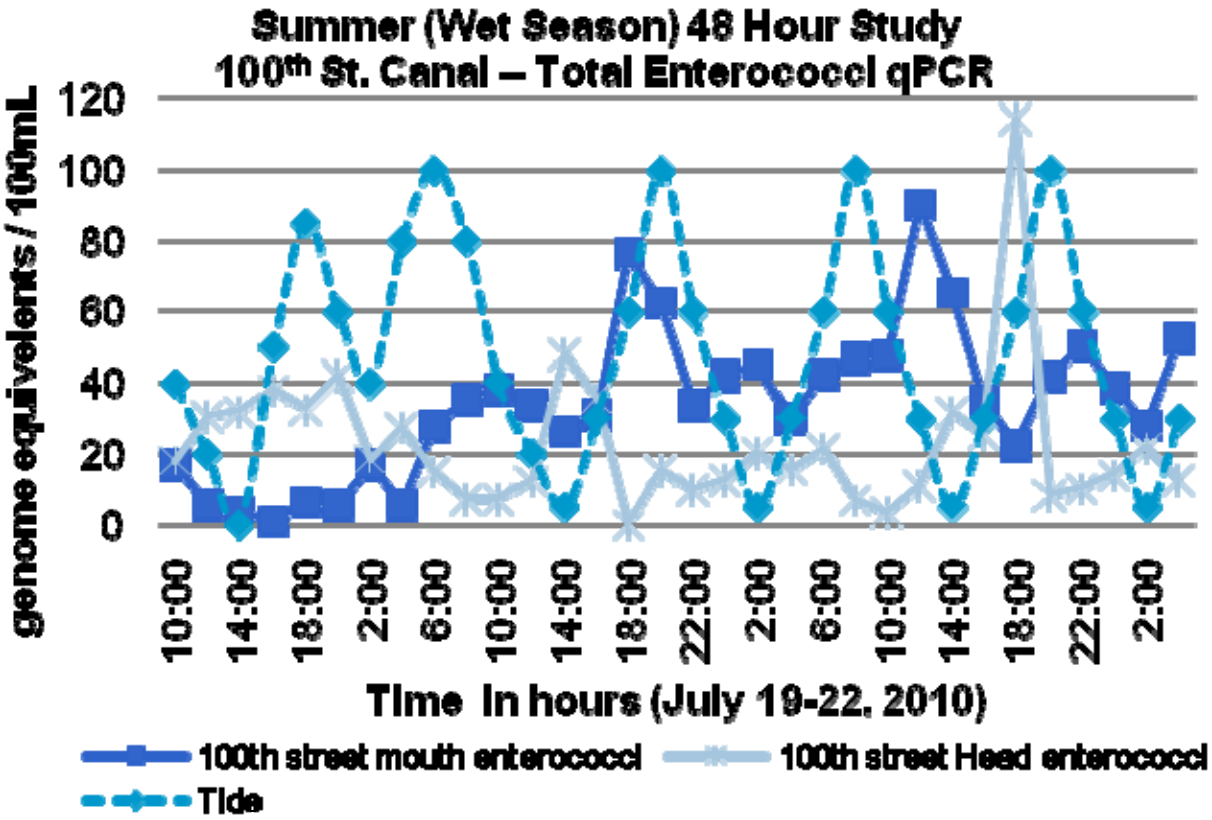

Figure 13: fluctuations of total enterococci over time at head and mouth of 100th Street Canal during the Summer (wet season) 48 hour diurnal study (July 19-22, 2010), as measured every 2 hours by the entero1 qPCR marker.

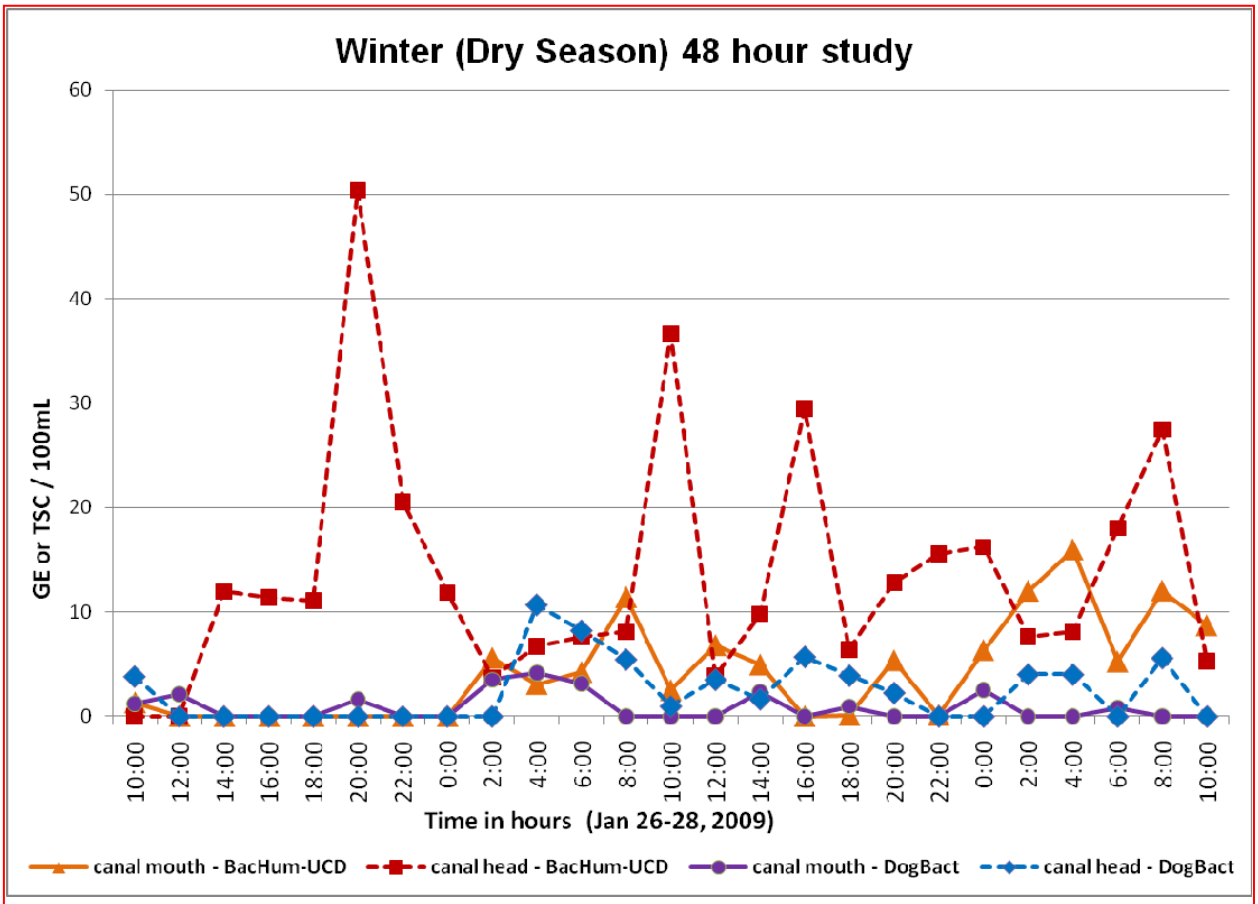

Figure 14: fluctuations of human source Bacteroidales and dog source Bacteroides over time at head and mouth of 100th Street Canal during the Winter (dry season) 48 hour diurnal study (Jan 26-28, 2009), as measured every 2 hours by the BacHum-UCD marker and DogBact marker respectively (UCD in genome equivalent units and DogBact in target sequence copies)

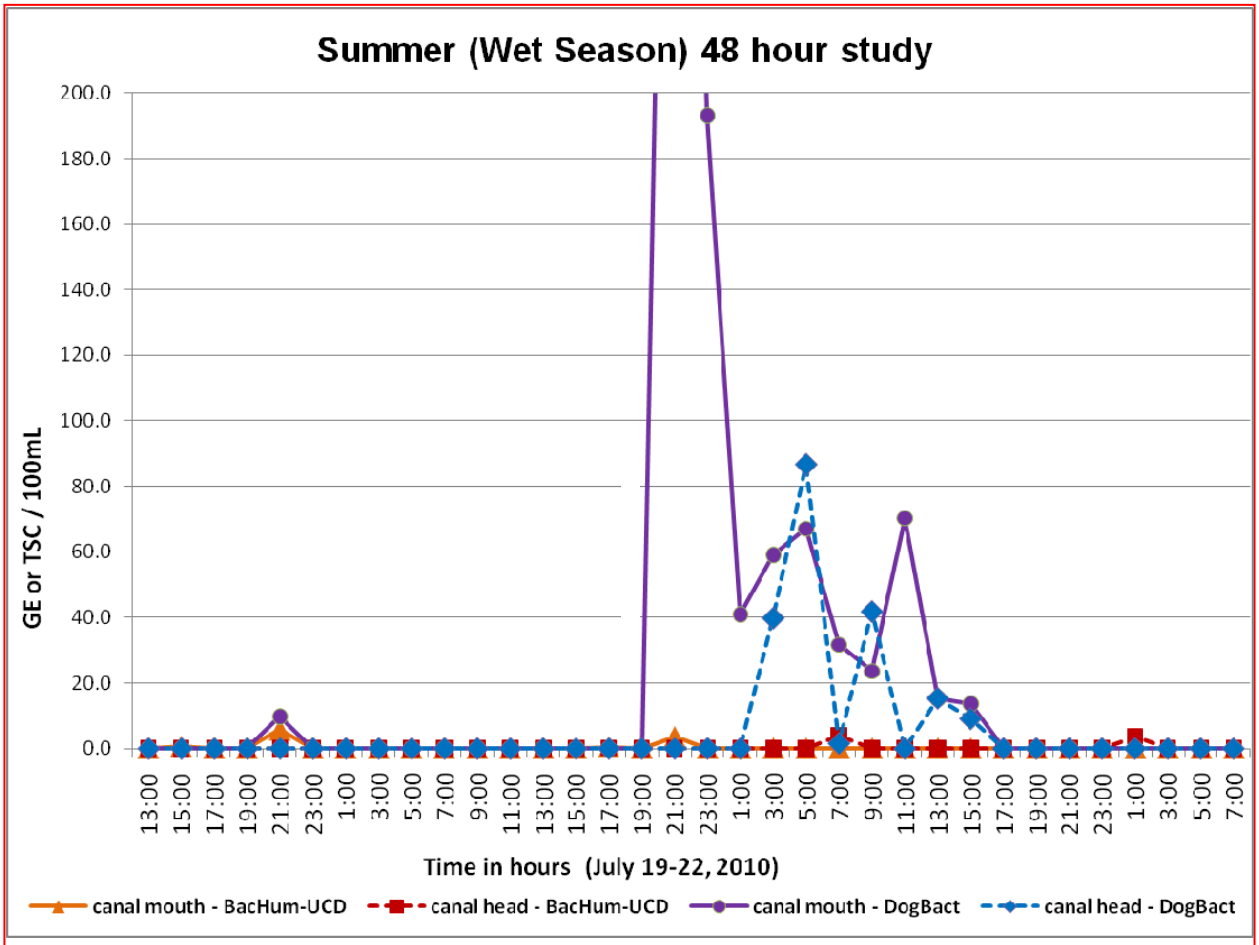

Figure 15: fluctuations of human source Bacteroidales and dog source Bacteroides over time at head and mouth of 100th Street Canal during the Summer (wet season) 48 hour diurnal study (July 19-22, 2010), as measured every 2 hours by the BacHum-UCD marker and DogBact marker respectively (UCD in genome equivalent units and DogBact in target sequence copies). Offscale value of DogBact for timepoint 21:00 = 516 TSC/100mL.

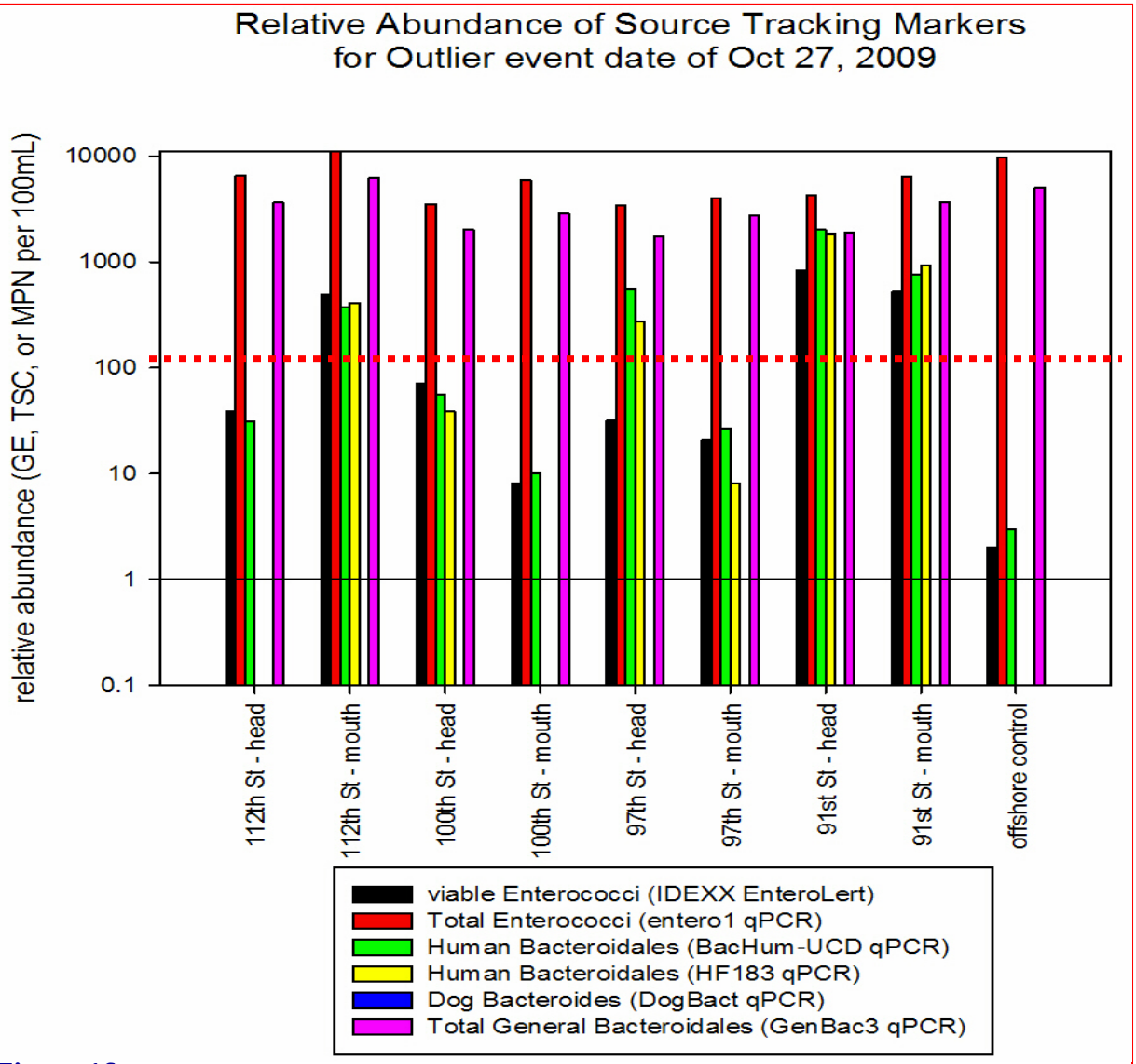

Figure 16: The sample date of Oct 27,2009 stood out as unusual during the course of the study for having extremely high levels of many fecal indicator source tracking markers at most of the sample sites. This date had the highest levels observed for multiple human source markers, and for enterococci qPCR markers. Both 91st St canal and 112th St canal showed exceedance of regulatory limits for viable enterococci. Extremely high levels of total enterococci as measured by qPCR were seen in all canals as well as the offshore control site, and very high levels of human Bacteroides markers by multiple assays were seen for the many of the canal sites. Values for this date represent high end outliers for most of the assays in the study data set. The 91st St septic control canal was positive for all human markers tested (except adenovirus). Interestingly, no significant dog marker was seen for any sample on this date, despite the very high levels of other markers. Units are in Most Probable Number (MPN) for viable enterococci by IDEXX EnteroLert, in Genome Equivalents (GE) for entero1, BacHum-UCD, HF183, and GenBac3 qPCR assays, and in Target Sequence Copies (TSC) for DogBact). Note that relative abundance is in log10 scale. The dotted red line indicates the regualorty exposure limit for enterococci for single grab samples (full body exposure, recreational waters)